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Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Sterilizing Action of Acids. (V).

Sterilizing action of dibasic fatty acids on Putrifactive bacteria, Bac. typhosus and Vib. cholerae.

By

Sogo TETSUMOTO

(Received October 31, 1933,)

Contents

Details of the sterilizing action of monobasic fatty acids are as reported previously.[1), 2)]

To know the sterilizing action of dibasic fatty acids on Staphylococcus pyogenes aureus, Proteus vulgaris, Hauser, Bac. typhosus and Vib. cholerae, I used the following acids.

(1) Reagents.

Table I.

Acid	Number of C atom	Rational formulae	Molecular weight	pH		
				N/10	*N*/100	*N*/1000
Oxalic	C_2	$\begin{matrix}CO_2H\\CO_2H\end{matrix} \cdot 2H_2O$	126.048	1.52	2.09	3.09
Malonic	C_3	$CH_2\begin{matrix}CO_2H\\CO_2H\end{matrix}$	104.048	2.04	2.64	3.2
Succinic	C_4	$(CH_2)_2\begin{matrix}CO_2H\\CO_2H\end{matrix}$	118.048	2.6	3.1	3.6
Glutaric	C_5	$(CH_2)_3\begin{matrix}CO_2H\\CO_2H\end{matrix}$	132.089	2.66	3.2	3.66
Adipinic	C_6	$(CH_2)_4\begin{matrix}CO_2H\\CO_2H\end{matrix}$	146.110	2.72	3.22	3.72
Pimelinic	C_7	$(CH_2)_5\begin{matrix}CO_2H\\CO_2H\end{matrix}$				
Suberinic	C_8	$(CH_2)_6\begin{matrix}CO_2H\\CO_2H\end{matrix}$	176.152	——	3.26	3.76
Azelainic	C_9	$(CH_2)_7\begin{matrix}CO_2H\\CO_2H\end{matrix}$	188.173	——	3.30	3.80
Sebacinic	C_{10}	$(CH_2)_8\begin{matrix}CO_2H\\CO_2H\end{matrix}$	202.194	——	——	3.78

(2) Sterilizing action at the same molecular concentration.

I made $N/10$, $N/100$ and $N/1000$ of aquoeus soltion by acids mentioned previously in Table I.

Results are mentioned in the following table.

Table II.

(1) Sterilizing action at $N/10$.

Acid	pH	Surviving period																					
		Staph. c. pyogen.						Prot. vulgar., H.						Bac. typhos.						Vib. chol.			
		m 2.5		10	20	h 2	3	m 1	2.5	5	60	90		m 1	5	10	90	h 2	m 1	2.5		
Oxalic	2.5	+	−	−	−	−	−	−	−	−	−	−		±	−	−	−	−	−	−		
Malonic	2.0	+	+	±	−	−	−	+	+	−	−	−		+	+	−	−	−	−	−		
Succinic	2.9	+	+	+	+	+	−	+	+	+	+	−		+	+	+	+	−	±	−		
Glutaric	2.7	+	+	+	+	+	−	+	+	+	+	−		+	+	+	+	−	±	−		
Adipinic	〃	+	+	+	+	±	−	+	+	+	+	−		+	+	+	±	−	±	−		
Control		+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		

(2) Sterilizing action at $N/100$.

Acid	pH	Surviving period																															
		Staph. c. pyog								Prot. valgar. H.									Bac. typhosus									Vib. choler.						
		m 45	60	h 6	9	12	24	36	48	m 15	20	30	60	h 3	9	12	18	20	m 30	45	h 2	3	6	12	24	m 1	2.5	5	10	20	30	60
Oxalic	2.1	±	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	−	−
Malon.	2.6	+	+	−	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−
Succin.	3.1	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−
Glutar.	3.2	+	+	+	+	+	+	−	−	+	+	+	+	+	±	−	−	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−
Adipin.	〃	+	+	+	+	+	±	−	−	+	+	+	+	+	−	−	−	+	+	+	+	+	+	±	−	+	+	+	+	±	−	−
Suberin.	3.3	+	+	+	+	−	−	−	−	+	+	+	+	−	−	−	−	+	+	+	+	+	−	−	−	+	+	+	+	−	−	−
Azelain.	〃	+	+	±	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	±	−	−	−	−
Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(3) Sterilizing action at $N/1000$.

Acid	pH	Surviving period																																	
		Staph. c. pyogen.								Prot. vulgar., H.									Bac. typhosus									Vib. choler.								
		h 6	9	12	24	36	48	72	2	h 3	6	9	12	24	36	48	3	h 6	9	12	24	36	48	60	m 10	20	30	45	60	90	h 2				
Oxal.	3.1	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−				

Malon.	3.2	+	+	±	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	−	−	−	−
Succin.	3.6	+	+	+	+	+	+	−	+	+	+	+	+	+	±	−	+	+	+	+	+	+	±	−	+	+	+	+	+	−
Glut.	3.7	+	+	+	+	+	±	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	−
Adip.	〃	+	+	+	+	+	−	−	+	+	+	+	+	−	−	−	+	+	+	+	+	±	−	−	+	+	+	+	+	−
Suber.	3.8	+	+	+	+	−	−	−	+	+	+	±	−	−	−	−	+	+	+	+	+	−	−	−	+	+	+	+	−	−
Azel.	〃	+	+	+	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+	+	−	−
Sebac.	〃	+	−	−	−	−	−	−	+	±	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	±	−	−	−	−
Control		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

From the results noted in Table II (1), (2), (3) we find the following facts.

At the same molecular concentration of dibasic fatty acids, oxalic acid (C_2) is the strongest, malonic acid (C_3) and sebacinic acid (C_{10}) follow to this respectively as concerning the sterilizing action.

According to the increase of the number of C atom from C_3 acid, the sterilizing power greatly decrease. Succinic acid (C_4) is the weakest of all among dibasic fatty acids. From adipinic acid (C_6) according to the increase of the number of C atom, the solubility of acids to water greatly diminishes, but the sterilizing action increases gradually. On this respect, the fact resembles the sterilizing action of monobasic fatty acids.[1]

Notice: $N/10$ of suberinic acid, azelainic acid and sebacinic acid, and $N/100$ of sebacinic acid are not made because each acid insoluble in water at the such concentration, so I did not study.

(3) Sterilizing action of anions of dibasic fatty acids.

To know the sterilizing action of anions of dibasic fatty acids I made aqueous solution of Na, Ca and NH_4 salt having the same anion of dibasic fatty acids. Concentration of salts of C_2 to C_6 acid are $N/100$, and those of C_8 to C_{10} acids are $N/1000$ respectively.

Results are as shown in table III (1), (2) and (3).

Table III.—Sterilizing action of anions of dibasic fatty acids.

(1) Sterilizing action of Na salts.

Na–	Concent. Nor.	Surviving period																
		Staph. pyogen.				Prot. vulgar.				Bac. typhos.					Vib. choler.			
		6d	7	8	9	4d	5	6	7	4d	5	6	7	8	3h	6	9	12
Oxalate	1/100	+	±	−	−	+	−	−	−	+	±	−	−	−	+	+	±	−
Malonate	〃	+	+	−	−	+	+	−	−	+	+	−	−	−	+	+	+	−
Succinate	〃	+	+	+	−	+	+	±	−	+	+	+	±	−	+	+	+	−
Glutarate	〃	+	+	+	−	+	+	±	−	+	+	+	−	−	+	+	+	−
Adipinate	〃	+	+	±	−	+	±	−	−	+	+	+	−	−	+	+	+	−

Suberinate	1/1000	+	+	±	−	+	±	−	−	+	+	±	−	−	+	+	+	−
Azelainate	〃	+	+	−	−	+	+	−	−	+	+	−	−	−	+	+	+	−
Sebacinate	〃	+	−	−	−	±	−	−	−	+	±	−	−	−	+	±	−	−
Control		+	+	+	−	+	±	−	−	+	+	±	−	−	+	+	+	+

(2) Sterilizing action of Ca salts.

Ca-	Concent. Nor.	Surviving period																
		Staph. pyogen.					Prot. vulgar.				Bac. typhos.					Vib. choler.			
		3d	6	7	8	9	3d	4	5	6	3d	4	5	6	7	2h	3	6	9
Malonate	1/100	+	+	±	−	−	+	+	−	−	+	+	+	−	−	+	+	±	−
Succinate	〃	+	+	+	−	−	+	+	+	−	+	+	+	±	−	+	+	+	−
Glutarate	〃	+	+	+	−	−	+	+	±	−	+	+	+	−	−	+	+	±	−
Adipinate	〃	+	+	±	−	−	+	+	±	−	+	+	+	−	−	+	+	−	−
Suberinate	1/1000	+	+	±	−	−	+	+	−	−	+	+	+	−	−	+	±	−	−
Azelainate	〃	+	+	−	−	−	+	+	−	−	+	+	−	−	−	+	±	−	−
Sebacinate	〃	+	−	−	−	−	+	−	−	−	+	−	−	−	−	+	−	−	−
Control		+	+	+	+	−	+	+	±	−	+	+	+	±	−	+	+	+	+

Notice: Ca-Oxalate is insoluble in water, so I did not study its sterilizing action.

(3) Sterilizing action of NH_4- salts.

NH_4-	Concent. Nor.	Surviving period															
		Staph. pyogen.				Prot. vulgar.				Bac. typhos.				Vib. choler.			
		7d	8	9	10	4d	5	6	7	5d	6	7	8	3h	6	9	12
Oxalate	1/100	+	−	−	−	+	±	−	−	+	+	−	−	+	+	±	−
Malonate	〃	+	+	−	−	+	+	−	−	+	+	+	−	+	+	±	−
Succinate	〃	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	−
Glutarate	〃	+	+	±	−	+	+	±	−	+	+	+	−	+	+	+	−
Adipinate	〃	+	+	±	−	+	+	±	−	+	+	±	−	+	+	+	−
Suberinate	1/1000	+	+	−	−	+	+	−	−	+	+	−	−	+	+	±	−
Azelainate	〃	+	±	−	−	+	+	−	−	+	−	−	−	+	+	−	−
Sebacinate	〃	±	−	−	−	+	−	−	−	±	−	−	−	+	+	−	−
Control		+	+	−	−	+	±	−	−	+	±	−	−	+	+	+	+

From the results noted in table III, we find the following facts. Salts of C_3 to C_8 acids have no sterilizing action but salts of C_2, C_9 and C_{10} acids have weak sterilizing action. Among salts of Na, Ca and NH_4, Ca salts have a somewhat short surviving period, and NH_4 salts have a somewhat long surviving period.

These salts are all in neutral aqueous solution and have the same anions as acids. From these facts we deduce the following: Anions of C_3 to C_6 acids have no sterilizing action. Anions of C_2, C_9 and C_{10} acids have weak sterilizing action respectively.

(4) Comparison of sterilizing action between dibasic and monobasic fatty acid of the same molecular concentration.

Saturated monobasic and unsaturated monobasic fatty acids series have many reagents. According to the number of C atom, these reagents differ from each other about their physical and chemical properties and also about their sterilizing action.

Accordingly if we compare the sterilizing action of these 3 series of acids, we must compare acids having the same number of C atom, at the same pH of acids.

I compare the sterilizing action between saturated and unsaturated monobasic fatty acids and dibasic fatty acids having the same number of C atom, at the same molecular concentration.

The results are as shown in the following table.

Table IV.—Comparison of the sterilizing action between saturated and unsaturated monobasic fatty acids and dibasic fatty acids.

(1) Results at *N*/100.

Number of C	Acid	pH	Surviving period							
			Staph. pyog. (m)		Prot. vulgar. (m)		Bac. typhos. (m)		Vib. choler (m)	
C_2	Acetic	3.4	540	+	120	+	180	±	15	+
	Oxalic	2.1	45	±	15	+	20	+	2.5	+
C_3	Propionic	3.4	540	+	120	+	180	±	15	+
	Acrylic	3.1	363	+	60	+	120	±	10	+
	Malonic	2.6	60	+	30	+	45	+	5	+
C_4	Butyric	3.4	540	+	120	+	180	±	15	+
	Isobutyric	〃	720	+	180	+	180	+	20	+
	Crotonic	3.3	540	±	120	±	120	+	15	+
	Succinic	3.1	1440	+	720	+	720	+	30	+
C_5	Valeric	3.4	540	+	120	±	120	+	15	+
	Isovaleric	〃	720	±	180	±	180	+	20	±
	Glutaric	3.2	1440	+	720	±	720	+	20	+
C_6	Caproic	3.4	180	±	60	+	90	+	2.5	±
	Isocaproic	〃	360	+	120	±	120	+	2.5	+
	Adipinic	3.2	1440	±	550	+	720	+	20	±

(2) Results at *N*/1000 and saturated aqueous solution.

Number of C	Acid	pH	Surviving period							
			Staph. pyogen. (m)		Prot. vulgar. (m)		Bac. typhos. (m)		Vib. choler. (m)	
C_2	Acetic	3.9	2160	±	720	+	1440	+	60	+
	Oxalic	3.1	360	+	120	+	180	+	10	+
C_3	Propionic	3.9	2160	±	720	+	1440	+	60	+
	Acrylic	3.6	720	+	540	+	720	±	45	±
	Malonic	3.2	720	±	360	+	540	+	20	+
C_4	Butyric	3.9	2160	±	720	+	1440	+	60	+
	Isobutyric	〃	2880	+	1440	±	2160	+	90	+
	Crotonic	3.8	1440	+	720	+	1440	±	60	+
	Succinic	3.6	2880	+	2160	+	2880	±	90	+
C_5	Valeric	3.9	144)	+	720	+	1440	±	45	±
	Isovaleric	〃	2880	±	1440	±	2160	+	60	+
	Glutaric	3.7	2880	±	1440	+	2160	+	90	+
C_6	Caproic	3.9	1440	±	540	±	540	±	10	+
	Isocaproic	〃	2160	+	720	±	720	+	30	±
	Adipinic	3.7	〃	+	720	+	2160	±	90	±
C_8	Caplylic	4.8	20	±	2.5	±	5	±	1	−
	Suberinic	3.8	1440	±	540	±	1440	+	60	±
C_9	Perargonic	4.8	20	±	2.5	±	5	±	1	−
	Azelainic	3.8	720	+	360	+	540	+	45	+
C_{10}	Capric	4.8	20	±	2.5	±	5	±	1	−
	Sebacinic	3.8	360	+	180	±	180	+	20	+

Caprylic, pelargonic and capric acids are examined at saturated solution at 20°C.

From the results noted in table 4, we find the following facts. If we compare the sterilizing action at the same molecular concentration of acids having the same number of C atom, dibasic fatty acids are strongest, unsaturated monobasic fatty acids are next, and saturated monobasic fatty acids are weakest.

The fact is chiefly due to pH. At C_4 acids, unsaturated monobasic fatty acid is the strongest, saturated acid is the next and dibasic fatty acid is the weakest concerning the sterilization. From C_5 acid, according to the increase of the number of C atom, the sterilizing action of monobasic fatty acids are stronger than those of dibasic fatty acids. pH of monobasic fatty acids is higher than that of dibasic fatty acids, and that the sterilizing action of monobasic fatty acids are stronger than dibasic acids. The cause is due to the effect

of undissociated molecule of each acids.

(5) Comparison of sterilizing action between dibasic and monobasic fatty acids at the same pH.

To find the sterilizing action at the same pH of dibasic fatty acids, and to compare the sterilizing action at the same pH of monobasic fatty acids, I made solution of pH 3.0 with C_2 to C_5 acids, pH 4.0 with C_2 to C_6 acids and pH 4.8 with C_8 to C_{10} acids, and compared them at the same number of C atom. Results are noted in table V (1), (2).

Table V.

(1) Comparison at pH 3.0.

Number of C	Acid	Normal	Surviving period							
			Staph. pyogen. (m)		Prot. vulgar. (m)		Bac. typhos. (m)		Vib. choler. (m)	
C_2	Acetic	1/20	120	+	45	+	90	+	1	+
	Oxalic	1/900	360	+	120	+	180	±	10	+
C_3	Propion.	1/20	120	+	45	+	90	+	1	+
	Acrylic	〃	360	+	90	±	180	±	5	+
	Malonic	1/800	〃	+	180	±	〃	±	10	+
C_4	Butyric	1/20	120	+	45	+	90	+	1	+
	Isobutyr.	〃	180	+	60	+	120	+	2.5	+
	Croton.	1/40	360	±	90	±	〃	+	5	±
	Succinic	1/80	1440	±	540	+	720	±	20	+
C_5	Valeric	1/20	120	+	45	+	90	+	1	−
	Isovaler.	〃	180	+	60	+	120	+	2.5	+
	Glutaric	1/60	720	+	360	+	540	+	15	+

(2) Comparison at pH 4.0 and pH 4.8.

Number of C	Acid	Concent. Nor.	Surviving period							
			Staph. pyogen. (m)		Prot. vulgar. (m)		Bac. typhos. (m)		Vib. choler. (m)	
C_2	Acetic	1/2000	2160	+	720	+	1440	+	60	+
	Oxalic	1/9000	5760	+	2160	+	2880	+	120	+
C_3	Propion.	1/2000	2160	+	720	+	1440	+	60	+
	Acrylic	1/8000	4320	+	2160	+	2880	+	120	+
	Maronic	1/8000	〃	+	〃	+	〃	+	90	+
C_4	Butyric	1/2000	2160	+	720	+	1440	+	60	+
	Isobutyr.	〃	2880	±	1440	+	2160	+	90	+
	Croton.	1/4000	4320	+	2160	±	2880	±	〃	+
	Succinic	1/8000	8640	+	4320	+	5760	±	180	±

C_5	Valeric	1/2000	2160	+	1440	+	1440	+	60	+
	Isovaler.	"	2880	±	2160	±	2160	+	90	+
	Glutur.	1/6000	7200	±	2880	+	4320	±	120	+
C_6	Caproic	1/2000	1440	+	540	+	720	±	2.5	+
	Isocaproic	1/2000	2160	+	720	+	1440	+	60	+
	Adipin.	1/6000	5760	+	2160	+	4320	±	120	+
C_8	Caplylic	1/1000	20	+	2.5	±	5	+	1	−
	Suberin.	1/4000	4320	±	1440	+	2160	+	90	+
C_9	Pelargonic	1/1000	20	±	2.5	±	5	+	1	−
	Azelainic.	1/4000	2160	+	720	+	1440	+	90	±
C_{10}	Capric	1/1000	20	±	2.5	±	5	+	1	−
	Sebacinic	1/4000	720	±	350	±	720	±	45	±

In table V, line of concentration shows the concentration of each acids due to pH 3.0, pH 4.0 or pH 4.8.

From the results noted in Table V (1) and (2), we find the following. If we compare the sterilizing action of acids of C_2 to C_{10} at the same pH, we find that saturated monobasic fatty acids have the strongest sterilizing action, unsaturated fatty acids have the next and dibasic fatty acids have the weakest sterilizing action.

The fact is distinctly according to the increase of C atom. If we compare the molecular concentration of each acids, concentration of dibasic fatty acids is smaller than those of monobasic fatty acids.

By this fact we find that difference of sterilizing action of the same pH solution of fatty acids, due to the difference of molecular concentration of each acids.

Summary.

I studied the sterilizing action of dibasic fatty acids $C_nH_{2n} \cdot (Co_2H)_2$ on putrifactive bacteria, Bac. typhosus and Vib. cholerae, and also compared the sterilizing action between dibasic and monobasic fatty acids.

Results are as follows:

(1) At the same molecular concentration, oxalic acid (C_2) is the strongest, malonic acid (C_3) is the next, and from malonic acid the sterilizing power suddenly greatly diminishes. From C_6 acid the sterilizing action of acids gradually increases, according to the increase of the number of C atom.

(2) Anions of C_2 to C_6 acids have no sterilizing action. According to the increase of the number of C atom from C_6 acid the sterilizing power of anions gradually increases, but its action is very weak.

(3) Among salts of Na, Ca and NH_4, Ca salts have a somewhat weak

sterilizing power, and NH_4 salts have a somewhat long surviving period for bacteria.

(4) At the same molecular concentration of acids having the same number of C atom, from C_2 to C_3 acids, dibasic fatty acid is the strongest, unsaturated monobasic fatty acid is the next, and saturated monobasic fatty acid is the weakest. The fact is chiefly due to pH. From C_5 acid, according to the increase of the number of C atom, the sterilizing action of monobasic fatty acids are stronger than those of dibasic fatty acids. The fact due to the effect of undissociated molecules of each acids.

(5) At the same pH, sterilizing action of saturated monobasic fatty acid is the strongest, unsaturated monobasic fatty acid is the next and didasic fatty acid is the weakest of all. The fact is due to the difference of molecular concentration of each acids.

(6) There is no special relation between the number of CO_2H group and the strength of sterilizing action.

Refference.

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Bombilupeol, a Colorless Crystallizable Constituent of the Alcoholic Extract of the Domestic Cocoon, (Résumé)

By

Masami Oku

(From the Chemical Laboratory of Gunze Raw Silk Mfg, Co. Ltd., Ayabemati, Kyoto-fu, Japan.)

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In the course of my investigation regarding the natural coloring matters of the yellowish green domestic cocoon named "Seihaku", a colorless substance which gave the color reactions of phytosterol was isolated from the ether soluble fraction of the cold alcoholic extract of the cocoon layer in the pure crystallized form and found to be identical with lupeol by its molecular formula and other properties.

The auther decided to give the name of "Bombilupeol" to the substance because no one has yet isolated it not only from domestic cocoon (Bombyx

mori), but also from animal origin.

Experimental.

The cold 70% ethyl alcoholic extract of the cocoon layer was condensed and fractionated into two parts, namely, the ether soluble in which bombilupeol dissolved and the aqueous in which a great part of the yellowish green pigment was dissolved. From the none-saponifiable fraction of the ether soluble part, the pure substance was isolated after repeating the recrystallization from methanol, using animal charcoal. The yield of the pure crystal was about 0.01% of the cocoon layers.

The substance crystallized out as colorless, needle shaped crystals, sometimes forming radiant colonies with silky appearance. When crystallized out from ether, it formed needles arranged in sheaves. The pure crystal melted sharply at 213° (corr.) and showed no depression of the melting point by mixing in it the lupeol which was isolated from the bark of "Sikeihi". From the results of analysis the molecular formula was given as $C_{30}H_{50}O$ with one double bond. The rotation in chloroform solution was estimated as $[\alpha]_D^{25} = +27.50°$. The substance was insoluble in water, acids or alkalies, sparingly soluble in cold alcohol but soluble in hot alcohol and readily soluble in cold ether, chloroform, benzene and petroleum ether and gave, after long elapse of time, the color reactions of Liebermann-Burchard and others, which being characteristic to phytosterol. On treatment with acetic acid anhydride and benzoyl chloride, the substance was converted respectively into acetate (M. P. 216°) and benzoate (M. P. 270°).

The trial to detect the bombilupeol in the other species of domestic cocoon, eg., yellowish, pale green and white, gave negative results, but a minute quantities of substances gave color reactions of phytosterol was isolated, leaving its further examination in future.

The origin of bombilupeol of the yellowish green cocoon can be presumed as deriving from the mulberry leaves on which silk warm feeds but its detection in the leaves must be carried out by further experiment.

Über die Bildung des Harnstoffs aus Uraminosäuren, Hydantoinen und aus Eiweisskörpern durch Einwirkung von Enzymen (Reduktasen) in neutraler Lösung.

Von

Mitsunori WADA.

(*Agrikultur-Chemisches Institut der Tokyo Kaiserl. Universität, Komaba, Tokyo,*)

(Eingengangen am 17, Januar 1934)

In der vorigen Arbeit[1] hat der Verfasser mitgeteilt, dass der Harnstoff aus Citrullin, Prolysin, verschiedenen Hydantoinen, sowie aus Eiweisskörpern durch Einwirkung von Schwefelwasserstoff in schwach alkalischer Lösung gebildet wird.

In weiterer Fortsetzung dieser Arbeit hat der Verfasser beobachtet, dass in Milch, Blut, Pankreas oder Leber ein gewisses Enzym vorhanden ist, welches die Fähigkeit besitzt aus den obengenannten Substanzen Harnstoff abzuspalten. Es sei nur erwähnt dass in frischer Milch kleine Menge Harnstoff ursprünglich vorhanden ist. So wurde in einem Versuche, aus 1850 ccm frischer Milch 0.85 g roher Harnstoff isoliert. Durch Sterilisieren bei 100° nimmt er aber nicht zu. In Blut, Pankreas oder Leber lässt sich auch etwas Harnstoff nachweisen.

Wenn man eine bestimmte Menge Citrullin, Prolysin oder Hydantoinen in frischer Milch zusetzt und 1～2 Stunden bei 60～70° stehen lässt, so beobachtet man immer die Vermehrung des Harnstoffs. Wenn aber anstatt frischer Milch bei 100° sterilisierte Milch verwendet wird, so findet keine Vermehrung des Harnstoffs statt. Daraus kann man schliessen dass das Enzym in Milch, aus Citrullin, Prolysin usw. Harnstoff abgespalten hat.

Dieselbe Versuche wurden auch mit denselben Resultate mit Blut, Pankreas und Leber wiederholt.

Obgleich die Natur des betreffenden Enzyms noch nicht klar ist, nehmen wir doch an, dass es sich um eine Reduktase handelt, weil die Bildung des Harnstoffs mit der reduzierenden Wirkung der Milch, Blut usw. beinahe parallel geht.

Experimenteller Teil.

(1) 1850 ccm frische Milch wurden im Vakuum bis zum Trocknen eingedampft, und mit Methylalkohol extrahiert. Die Methylalkoholishe Lösung wurde wieder eingedampft und nach Behandlung mit Petroleumäther, mit

Äther wiederholt ausgezogen. Beim Verdampfen des Äthers schied sich der Harnstoff kristallinisch aus. Der letztere war in Wasser und Alkohol löslich und gab die P. Ehrlichsche sowie die Schiffsche Reaktion sehr stark. Durch Einwirkung von Urease in wässeriger Lösung wurde er in Ammoniak gespalten. Zur weiteren Identifizierung wurde er in Nitrat, Oxalat und in Dixanthyl-derivat verwandelt. Die Ausbeute an rohem Harnstoff betrug 0,85 g.

(2) 0,0512 g Citrullin wurden in 25 ccm frischer Milch gelöst, mit soviel *n*/10 Kalilauge versetzt bis die Lösung gegen Phenolrat neutral reagierte und eine Stunde auf dem Wasserbade bei 65° erwärmt. Nach dem Erkalten wurde der gebildete Harnstoff in Ammoniak verwandelt, indem die Lösung mit 1 ccm 10%iger Urease Lösung versetzt, mit wenig flüssigem Paraffin überschichtet und bei Zimmertemperatur stehen gelassen wurde. Nach 30 Minuten wurden das dadurch gebildete Ammoniak in bekannter Weise mit *n*/10 Schwefelsäure titriert.

In dieser Weise wurde gefunden:

	n/10 H_2SO_4 verbraucht zur Titration (ccm)	Berechnet für Harnstoff (mg)	% der Theorie
1) 25 ccm Milch + 0,0512 g Citrullin	4,2	11,735	
2) 25 ccm Milch ohne Zusatz von Citrullin	2,7	7,544	
3) NH_3 gebildet aus Citrullin (1−2)	1.5	4,191	23,86

Im folgenden werden die Resultate der in analoger Weise mit verschiedenen Substanzen ausgeführten Versuche tabellarisch zusammengestellt:

	n/10 H_2SO_4 verbraucht zur Titration (ccm)	Berechnet für Harnstoff (mg)	% der Theorie
1) 25 ccm frischer Milch	2,7	7,544	
2) " sterilizierte Milch	2,7	7,544	
3) " frischer Milch nach 1 Tag	3,0	8,382	
4) " " " " 1 Woche	3,7	10,337	
5) " sterilizierte Milch + 0,1024 g Carbamyl-alanin	2,7(2,7+0,0)	0,0	0,0
6) " " " + 0,1116 g Phenyl-alaninhydantoin	2,7(2,7+0,0)	0,0	0,0
7) " frischer Milch + 0,0512 g Citrullin	4,2(2,7+1,5)	4,191	23,86
8) " " " + 0,1010 g Carbamylleucin	4,5(2,7+1,8)	5,029	14,44
9) " " " + 0,1003 g Carbamylphenyl-alanin	5,2(2,7+2,5)	6,985	24,13
10) 50 ccm Milch (nach 1 Woche) + 0,0328 g Carbamylphenylalanin (erwärmt 2 St. 30 Min.)	7,8(3,7×2+0,4)	1,118	11,81
11) 25 ccm Milch + 0,0996 g Prolysin	3,8 2,7+1,1)	3,073	11,05

12)	25 ccm Milch + 0,10036 g Leucinhydantoin	3,4(2,7+0,7)	1,956	5,07
13)	" " + 0,10022 g Phenylalaninhydantoin	3,3(2,7+0,6)	1,676	5,30
14)	" " + 4,0 ccm Ovalbumin	3,4(2,7+0,7	1,956	
15)	" " + 3,0 g Gelatine	3,8(2,7+1,1)	3,074	

1 ccm H_2SO_4 = 4,561 mg H_2SO_4 = 2.794 mg Harnstoff

		H_2SO_4 (ccm) verbraucht zur Titration	Berechnet für Harnstoff (mg)	% der Theorie
1)	25 ccm Blutserum des Pferdes	4,6	12852	
2)	" " (erhitzt 30 Minuten bei 100°)	4,6	12852	
3)	" " (" " " " ") + 0,1154 g Carbamylphenylalanin	4,6 (4,6+0,0)	0,0	0,0
4)	25 ccm Blutserum + 0,1002 g Carbamylleucin	5,1 (4,6+0.5)	1,397	4,04
5)	" " + 0,1016 g Carbamylphenylalanin	5,5 (4,6+0,9)	2,515	8,58
6)	" " + 0,0996 g Prolysin	6 5 (4,6+1,9)	5,308	19,09
7)	" " + 0,1024 g Leucinhydantoin	6,3 (4,6+1,7)	4,749	12,06
8)	" " + 0,1006 g Phenylalaninhydantoin	6,2 (4,6+1,6)	4,470	14,07
9)	" " + 2,0 ccm Ovalbumin	5,0 (4,6+0,4)	1,118	
10)	" " + 1,5 g Gelatine	5,0 (4,6+0,4)	1,118	
1)	10 ccm Pankreassaft*	1,5	4,191	
2)	" " (erhitzt 30 Minuten bei 100°)	1,5	4,191	
3)	" " (" " " " ") + 0,1075 g Carbamylphenylalanin	1,5 (1,5+0,0)	0,0	0,0
4)	10 ccm Pankreassaft + 0,0996 g Prolysin	2,4 (1,5+0,9)	2,515	9,04
5)	" " + 0,10036 g Leucinhydantoin	2,3 (1,5+0,8)	2,235	5,80
6)	" " + 0,10022 g Phenylalaninhydantoin	2,4 (1.5+0,9)	2,515	7,94
7)	10 ccm Pankreassaft + 4,0 ccm Ovalbumin	1,8 (1,5+0,3)	0,838	
8)	" " + 3,0 g Gelatine	1,9 (1,5+0,4)	1,118	
1)	10 ccm Lebersaft*	1,2	3,352	
2)	" " (erhitzt 30 Minuten bei 100°)	1,2	3,352	
3)	" " (" " " " ") + 0,1035 g Carbamylphenylalanin	1,2 (1,2+0,0)	0,0	0,0
4)	10 ccm Lebersaft + 0,0996 g Prolysin	2,0 (1,2+0,8)	2,235	8,04
5)	" " + 0,10036 g Leucinydantoin	1,9 (1,2+0,7)	1,955	5,07
6)	" " + 0,10022 g Phenylalaninhydantoin	1,9 (1,2+0,7)	1,955	6,18
7)	10 ccm Lebersaft + 4,0 ccm Ovalbumin	1,5 (1,2+0,3)	0,838	
8)	" " + 3,0 g Gelatine	1,6 (1,2+0,4)	1,118	

* 500 g Subst. extrahiert mit 100 ccm H_2O bei 70°.

Man sieht dass der Harnstoff aus verschiedenen Substanzen gebildet worden ist. Die Reaktion verlief aber nicht quantitativ, so dass die erhaltenen Werte viel niedriger waren als man erwartete.

Im nachsten Versuche wurde dem Reaktionsgemisch etwas Formaldehyd zugesetzt und zwar in folgender Weise:

2000 ccm Milch wurden mit 16 ccm gesättigter Lösung der Citronensäure versetzt, der entstandene Niederschlag abfiltriert. 100 ccm des Filtrats wurden mit 0,1000 g Carbamylphenylalanin versetzt und mit $n/10$ Kalilauge bis zu schwachsauer Reaktion neutralisiert und nach dem Zusatz von 1 ccm käuflichem Formaldehyd bei 35° stehen gelassen. Nach einem Tage wurde der gebildete Harnstoff in obenerwähnter Weise in Ammoniak verwandelt und mit $n/10$ Schwefelsäure titriert.

Die Resultate sind wie folgendes:

	$n/10$ H_2SO_4 verbraucht zur Titration (ccm)	Berechnet für Harnstoff (mg)	% der Theorie
1) 100 ccm des Filtrats + 0,1000 g Carbamylphenylalanin	27,5	76,835	
2) 100 ccm des Filtrats ohne Zusatz von Carbamylphenylalanin	21,1	58,953	
3) NH_3 gebildet aus Carbamylphenylalanin (1−2)	6,4	17,882	62,00

Ferner wurde das obenerwähnte Filtrat im Vakumn bis zum Ein fünftel Teil eingedampft und 50 ccm davon wurden mit carbamylphenylalanin versetzt und nach dem Zusatz von 1 ccm Formaldehyd 24 Stunden bei 35° stehen gelassen. In diesem Fall erhielt man etwas bessere Resultate wie aus folgender Tabelle zu ersehen ist.

	$n/10$ H_2SO_4 verbraucht zur Titration (ccm)	Berechnet für Harnstoff (mg)	% der Theorie
1) 50 ccm des obenerwähnten Konzentrats + 0,1026 g Carbamylphenylalanin	26,2	73,202	
2) " " " " " + 0,1002 g Carbamylleucin	27,6	77,114	
3) " " " " " allein ohne Zusatz	17,5	48,895	
4) NH_3 gebildet aus Carbamylphenylalanin (1−3)	8,7	24,307	82,15
5) " " " Carbamylleucin (2−3)	10,1	28,219	81,68

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Untersuchungen über die Enzyme von Bombyx mori L. IV. Mitteilung.

Über die Phenolasen des Blutes.

Von

Kazuo YAMAFUJI.

(Aus dem Biochem. Institut der Landw. Abteilung der Kaiserl. Kyushu-Univ. zu Fukuoka, Japan.)

(Eingegangen am 5, Januar 1934)

Über die Fermente bei Wirbellosen sind veraltete zahlreiche Angaben vorhanden; moderne, quantitative Arbeiten aber sehr spärlich. Als Versuchstier ein Zuchtinsekt, die Seidenraupe, benutzend, hat der Verf. bereits einige Studien über mehrere Fermente des Verdauungssaftes und Blutes derselben angestellt[4].

Die physiologische Bedeutung der Desmolasen sowie die gegenseitigen Beziehungen zwischen ihren Teilfermenten sind heute noch nicht ganz klar. Es wurde in meiner vorhergehenden Mitteilung [5] gezeigt, dass die Tyrosinase und Katalase des Blutes von Bombyx mori besonders im Laufe von drei Entwicklungsperioden sehr auffallend sich verändern. Diese bemerkenswerten Tatsachen haben mich weiter zu Untersuchungen der anderen Oxydationsfermente, der Phenolasen, des Blutes des gleichen Insekts veranlasst.

I. Eigentliche oxydatische Wirkung.

1) Methodik:– Ich wählte die Rasse Japan-110-G für meine Versuche aus, die im Sommer 1933 aufgezogen wurde. Die Messung der Fermentmenge geschah durch die Oxydation von Guajacol. Zu diesem Zweck wurden 2 ccm 1 proz. Guajacollösung, 1 ccm M/3 Phosphatpufferlösung von Ph 6.6, 6 ccm Wasser und 1 ccm 20 fach verdünntes Blut gemischt und 30 minuten lang bei 30° gehalten. Dann wurde die Versuchslösung bei 0° abgekühlt und sofort die entstandene Färbung kolorimetrisch bestimmt. Die in diesem Falle nötige Standardsfarbenskala kann nach der Beschreibung von Bansi und Ucko[2] hergestellt werden.

2) Optimale Ph.

Ph	Oxyd. G. mg	Oxydation, %	Ph	Oxyd. G. mg	Oxydation, %
5.3	5.83	29.15	6.8	6.48	32.40
5.9	6.25	31.25	7.0	6.48	32.40
6.2	6.73	33.65	7.4	6.25	31.25
6.6	7.00	35.00	8.0	6.03	30.15

G = Guajacol.

3) Optimaltemperatur.

Temp.	Oxyd. G. mg	Oxydation, %	Temp.	Oxyd. G. mg	Oxydation, %
0	0.35	1.75	30	1.25	6.25
10	0.87	4.35	40	1.51	7.55
20	1.04	5.02	50	1.27	6.35

4) Änderungen durch Hungerzustand bei den Raupen.

Hungerstunden	1	6	25	52
Oxyd. G. mg	0.45	1.27	0.23	0.13
Oxydation, %	2.25	6.35	1.15	0.65

5) Unterschied zwischen gesunden und kranken Raupen.

	Gesunde Raupen			Krank. Raup. (Nankabyo)		
	Gruppe, 1	Gruppe, 2	Mittel	Gruppe, 1	Gruppe, 2	Mittel
Oxyd. G. mg	0.21	0.18	0.20	0.47	0.42	0.45
Oxyd. %	1.05	0.90	1.00	2.35	2.10	2.25

6) Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori.

		♀		♂	
	Tage	Oxyd. G. mg	Oxyd. %	Oxyd. G. mg	Oxyd. %
Raupe (V. Lebensalter)	2	5.28	26.40	5.51	27.55
	4	0.45	2.25	2.39	11.95
	7	0.53	2.65	0.21	1.05
Einspinnen des Kokons	2	0.57	2.85	0.23	1.15
	4	0.44	2.20	0.20	1.00
Puppe	1	0.41	2.05	0.35	1.75
	3	0.82	4.10	0.37	1.85
	5	0.92	4.60	0.53	2.65
	7	1.21	6.05	0.37	1.85
	10	0.33	1.65	0.24	1.20
Schmetterling	1	15.40	77.06	16.22	81.10
	2	16.22	81.10	——	——

II. Peroxydatische Wirkung.

1) Methodik:– Die Untersuchungen wurden nach der Methode von Bach und Zubkowa [1] ausgeführt, und zwar mit einige Abänderungen. 2 ccm 1 proz. Guajacollösung, 1 ccm M/3 Phosphatpuffermischung von Ph 6.6, 1 ccm

0.2 proz. H_2O_2-Lösung, 5ccm Wasser und 1ccm 20fach verdünntes Blut wurden vermischt. Nach 30 Minuten langer Erwärmung auf 30° wurde die Versuchslösung auf 0° abgekühlt und die Abstufung der entstandenen Farbe kolorimetrisch bestimmt.

2) *Optimale Ph.*

Ph	Oxyd. G. mg	Oxyd. %	Ph	Oxyd. G. mg	Oxyd. %
5.3	5.83	29.15	6.8	5.93	29.65
5.9	6.03	30.15	7.0	5.93	29.65
6.2	6.03	30.15	7.4	5.85	29.15
6.6	6.14	30.70	8.0	5.64	28.20

3) *Optimaltemperatur.*

Temp.	Oxyd. G. mg	Oxyd. %	Temp.	Oxyd. G. mg	Oxyd. %
0	0.52	2.62	30	0.71	3.55
10	0.69	3.45	40	0.54	2.70
20	0.94	4.70	50	0.35	1.75

4) *Änderungen durch Hungerzustand bei den Raupen.*

Hungerstunden	2	7	24	51
Oxyd. G. mg	0.50	0.71	0.22	0.11
Oxydation, %	2.50	3.55	1.10	0.55

5) *Unterschiedzwischen gesunden und kranken Raupen.*

	Gesunde Raupen			Krank. Raup. (Nankabyo)		
	Gruppe, 1	Gruppe, 2	Mittel	Gruppe, 1	Gruppe, 2	Mittel
Oxyd. G. mg	0.15	0.13	0.14	0.41	0.35	0.38
Oxyd. %	0.75	0.65	0.70	2.05	1.75	1.90

6) *Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori.*

			♀		♂	
	Tage		Oxyd. G. mg	Oxyd. %	Oxyd. G. mg	Oxyd. %
Raupe	V Lebensalter	2	3.67	18.35	4.40	22.00
		4	0.50	2.50	0.54	2.70
		7	0.32	1.60	0.19	0.95
Einspinnen des Kokons	2		0.39	1.95	0.23	1.15
	4		0.41	2.05	0.26	1.30

Puppe	1	0.37	1.85	0.34	1.70
	3	0.73	3.65	0.35	1.75
	5	0.88	4.40	0.50	2.50
	7	1.16	5.80	0.35	1.75
	10	0.29	1.49	0.24	1.20
Schmetterling	1	16.22	81.10	19.35	96.75
	2	17.94	89.70	——	——

III. Diskussion.

Aus allen oben angeführten und in der dritten Mitteilung beschriebenen Versuchen ergibt es sich, dass verschiedene physiologische bezw. pathologische Verhaltnisse bei Bombyx mori in der Regel erhebliche Schwankungen des Gehaltes an Oxydationsfermenten im Blute bedingen. Vor allem können sehr bedeutende, verhältnismässig regelmässige Änderungen der Blutfermente bei Metamorphose als ein geeignetes Material für die Besprechung über die noch wenig bekannten, gegenseitigen Beziehungen zwischen den verschiedenen Oxydationsfermenten dienen.

Welcher Unterschied besteht zwischen den sogenannten wahren Oxydasen und Peroxydasen? Oppenheimer[5] fasste diese beiden enzymatischen Wirkungen auf die Polyphenole als Phenolasen zusammen. Verf. prüfte bei Bombyx mori die oxydatische und peroxydatische Wirkung des Blutes auf Guajacol und beobachtete, dass diese beiden Wirkungen in manchen Fällen gleichlaufend sich verändern.

Die Grenze zwischen der Wirkung der Phenolasen und der der Tyrosinase ist noch nicht entscheidend. Es wurde auch gefunden, dass Hunger und besonders Metamorphose bei Bombyx mori im allgemeinen parallele Steigerungen oder Senkungen dieser beiden Fermente des Blutes hervorbringen.

Ferner ist die Frage, ob in den Lebewesen ein Parallelismus zwischen dem Vorkommen der Peroxydase und der Katalase existiert oder nicht, vielfach erörtert worden. Bei Bombyx mori konnte ich solchen Parallelismus nicht beobachten. Es ist bemerkenswert, dass die Blutkatalase mit dem Beginn des Kokonspinnens der Larve bedeutend stärker wird, während die Blutperoxydase bei den Puppen sehr schwach ist.

Zusammenfassung.

(1) Die Phenolasen des Blutes von Bombyx mori wirken am besten bei Ph 6.6. Die Optimaltemperatur der eigentlichen oxydatischen Wirkung liegt bei 40° und diejenige der peroxydatischen bei 20°.

(2) Durch Hunger bei Raupen werden die Phenolasen erst etwas stärker und dann schwächer. Bei kranken Raupen (Nankabyosan) sind diese enzy-

matischen Wirkungen recht viel höher als bei gesunden.

(3) Die Wirkungen der Phenolasen sind im ersten Stadium des fünften Lebensalters der Larve beträchtlich aktiv, sinken dann aber allmählich. Während der Puppenperioden bleiben die Phenolasen bedeutend zurück und in den Tagen des Schmetterlingslebens vermehren sie sich rasch bis zu einem Maximum.

(4) Der Phenolasengehalt des Blutes im Larven- und Schmetterlingsstadium ist bei den Männchen etwas höher als bei den Weibchen, im Puppenstadium dagegen bei den Weibchen etwas höher als bei den Männchen.

(5) Es wurde in meinen Untersuchungen bestatigt, dass die Oxydationsfermente des Blutes im Laufe von drei Entwicklungsperioden von Bombyx mori sehr auffallend sich verändern, und zwar die Tyrosinase, eigentliche Oxydase und Peroxydase in der Regel miteinander gleichlaufend, aber die Katalase allein gegen dieselben vielmehr umgekehrt.

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Untersuchungen über die Enzyme von Bombyx mori L. V. Mitteilung.

Über die Saccharase und Maltase des Blutes.

Von

Kazuo YAMAFUJI.

(Aus dem Biochem. Institut der Landw. Abteilung der Kaiserl. Kyushu-Univ. zu Fukuoka, Japan.)

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In früheren Mitteilungen[1] hat der Verf. die merkwürdigen Beziehungen der verschiedenen physiologischen bezw. pathologischen Verhältnisse bei Bombyx mori zu den Schwankungen des Gehaltes an Oxdationsfermenten im Blute beschrieben. Da kein quantitativer Versuch über die Disaccharasen des Blutes der Seidenraupe existiert, wurde nun von demselben Autor ein Studium auf diesem Gebiet vorgenommen.

I. Über die Saccharase.

1) Methodik:– Zu dem nachstehend erwähnten Experimenten wurden drei Originalrassen der Seidenraupen, nämlich Japan-110-E, China-7-B und Europa-7-C, gebraucht. Die von mir angewandte Methodik für die quantitative Bestimmung der Fermentaktivität ist die folgende:

Das Totalvolumen der Proben betrug stets 13 ccm, wovon 10 ccm 10 proz. Saccharoselösung, 2 ccm M/3 Phosphatpufferlösung von Ph 6.8 und 1 ccm zweifach verdünntes Blut. Diese Reaktionsmischung, 24 Stunden bei 37° erwärmt, wurde mit 3 ccm 2 *N* Sodalösung versetzt und dann das Drehungsvermögen der erhaltenen Lösung polarimetrisch ermittelt.

2) Optimale Ph.

Ph	Drehungsabnahme, °	Saccharosespaltung, %	Ph	Drehungsabnahme, °	Saccharosespaltung, %
5.3	0.92	17.62	6.8	1.02	19.54
5.9	0.95	18.20	7.0	0.99	18.97
6.2	1.10	21.07	7.4	0.93	17.82
6.6	1.14	21.84	8.0	0.91	17.43

3) Unterschied zwischen gut gewachsenen und schlecht gewachsenen Raupen.

	Gut gewachs. Raupen		Schlecht gewachs. Raup.	
	♀	♂	♀	♂
Dreh.-abnahme, °	2.82	2.59	3.10	2.12
Sacch.-spalt, %	54.02	49.62	59.39	52.11

4) Änderungen durch Hungerzustand bei den Raupen.

	♀		♂	
Hungerstunden	Dreh.-abn. °	Sac.-sp. %	Dreh.-abn. °	Sac.-sp. %
2	1.69	31.23	1.16	22.22
24	1.47	28.16	1 15	22.03
48	1.22	23.37	0.96	18.37

5) Unterschied zwischen gesunden und kranken Raupen.

	Gesunde Raupen		Krank. Raup. (Nankabyo)	
	♀	♂	♀	♂
Dreh.-abnahme, °	1.70	1.48	0.97	1.27
Sacch.-spalt, %	32.57	28.35	18.58	24.33

6) *Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori.*

	Tage		♀ Dreh.-abn. °	♀ Sac.-sp. %	♂ Dreh.-abn. °	♂ Sac.-sp. %
Raupe	V. Lebensalter	3	3.87	74.14	3.81	72.99
		5	3.47	66.48	3.40	65.13
		8	2.91	55.75	2.65	50.77
Einspinnen des Kokons	2		2.35	45.02	2.38	45.59
	4		2.41	41.00	2.60	49.81
Puppe	2		1.69	32.38	1.57	30.08
	7		1.32	25.29	1.21	23.18
	9		1.21	13.18	1.08	20.69
Schmetterling	1		1.08	20.69	—	—

7) *Unterschied zwischen drei Originalrassen der Seidenraupen.*

V. Lebensalter, Tage	Japan-110-E ♀ Sac.-sp. %	Japan-110-E ♂ Sac.-sp. %	China-7-B ♀ Sac.-sp. %	China-7-B ♂ Sac.-sp. %	Europa-7-C ♀ Sac.-sp. %	Europa-7-C ♂ Sac.-sp. %
3	74.14	72.99	19.54	17.62	43.49	41.59
5	66.48	65.13	31.23	22.22	35.63	36.40
Reife	55.75	50.77	32.57	28.35	35.82	42.15

II. Über die Maltase.

1) *Methodik*:– Die Versuche erfolgten mittels folgendem Verfahren. Zur Mischung der 10 ccm 5 proz. Maltoselösung und der 2 ccm M/3 Phosphatpufferlösung von Ph 6.8 wurde 1 ccm Blut hinzugefügt und während 24 Stunden bei 37° stehen gelassen. Dann wurde die Reaktion durch 3 ccm 2 *N* Sodalösung sistiert und der Drehungsgrad der erhaltenen Lösung beobachtet.

2) *Optimale Ph.*

Ph	Drehungsabnahme, °	Maltosespaltung, %	Ph	Drehungsabnahme, °	Maltosespaltung, %
5.3	0.08	3.51	6.8	0.13	5.70
5.9	0.06	2.63	7.0	0.12	5.26
6.2	0.05	2.19	7.4	0.10	4.39
6.6	0.15	6.40	8.0	0.07	3.07

3) Unterschied zwischen gut gewachsenen und schlecht gewachsenen Raupen.

	Gut gewachsene Raupen		Schlecht gew. Raupen	
Dreh.-abnahme, °	0.22	0.18	0.23	0.20
Malt.-spalt. %	9.65	7.89	10.09	8.77

4) Änderungen durch Hungerzustand bei den Raupen.

Hungerstunden	Dreh.-abn. °	Malt.-sp. %	Dreh.-abn. °	Malt.-sp. %
2	0.23	10.09	0.21	9.21
24	0.17	7.46	0.19	8.33
48	0.20	8.77	0.22	9.65

5) Unterschied zwischen gesunden und kranken Raupen.

	Gesunde Raupen		Krank. Raup. (Nankabyo)	
Dreh.-abnahme, °	0.27	0.22	0.28	0.24
Malt.-spalt. %	11.84	9.65	12.28	10.53

6) Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori.

	Tage		Dreh.-abn. °	Mal.-sp. %	Dreh.-abn. °	Mal.-sp. %
Raupe	V. Lebensalter	3	0.09	3.95	0.07	3.07
		5	0.07	3.07	0.06	2.63
		8	0.23	10.09	0.19	8.33
Einspinnen von Kokons	2		0.26	11.40	0.28	12.28
	4		0.38	16.67	0.24	10.53
Puppe	2		0.20	8.77	0.18	7.89
	7		0.14	6.14	0.10	4.39
	9		0.10	4.39	0.08	3.51
Schmetterling	1		0.36	15.79	——	—

7) Unterschied zwischen drei Originalrassen der Seidenraupen.

V. Lebensalter, Tage	Japan-110-E				China-7-B				Europa-7-C			
	♀ Mal.-sp.	%	♂ Mal.-sp.	%	♀ Mal.-sp.	%	♂ Mal.-sp.	%	♀ Mal.-sp.	%	♂ Mal.-sp.	%
3	3.95		3.07		9.21		7.46		5.70		3.95	
5	3.07		2.63		10.09		9.21		7.46		3.07	
Reife	10.09		8.33		8.77		9.65		14.35		9.65	

III. Diskussion.

Bei höheren Tieren scheinen im allgemeinen die Blutfermente in der normalen Assimilation der Nahrungsmittel und bei dem Abbau der resorbierten Stoffe einehöchst geringe Rolle zu spielen. Bei niederen Tieren, wie Bombyx mori, müssen aber die Verhältnisse etwas andere sein.

Die in vorangegangenen Untersuchungen[2] über die Verdauungs- und Blutfermente gewonnenen Ergebnisse drängen zu der Annahme, dass die Hydrolasen des Blutes bei der Seidenraupe an der Verdauung bezw. am Stoffwechsel ein wichtigen Anteil nehmen.

Die in der vorliegenden Arbeit mitgeteilten Versuche dürften die Richtigkeit dieser Voraussetzung bestätigen.

Trotzdem das Maulbeerblatt eine ziemliche Menge Rohrzucker enthält, ist im Digestionssafte der Seidenraupe keine Saccharase vorhanden. Eine sehr schwache Saccharase findet sich in der Darmwand, aber eine recht viel starkere im Blut derselben. Durch Hunger vermindert sich bei der Larve die Blutsaccharase. Dasselbe Ferment wird vom Tage des Einspinnens in den Kokon bis zu den Tagen des Schmetterlings allmählich verringert.

Auf Grund dieser Tatsachen dürfte der Schluss gerechtfertigt sein, dass die Blutsaccharase bei Bombyx mori an der Aufspaltung des Rohrzuckers der Nahrung beteiligt ist.

Bekanntlich ist in frischem Maulbeerblatt Stärkemehl in bedeutender Menge enthalten, während der Malzzucker durch die Fermente des Digestionskanals der Seidenraupe kaum gespalten werden kann. Im Blute derselben kommt indessen eine schwache Maltase vor. Eine gewaltige Vermehrung der Glykogenvorräte findet im fünften Lebensalter der Larve statt, aber eine allmählige Verminderung derselben in den Puppen- und Schmetterlingsperioden von Bombyx mori. Die Wirkung der Blutmaltase ist in den Tagen der Kokonspinnens der Raupe am stärksten, wird im Laufe der Puppenperiode von Tag zu Tag schwacher und dann in der Periode des Schmetterlingslebens wieder gesteigert. Innerhalb der zwei Hungertage bei der Larve verändert sich die Blutmaltase nicht wesentlich.

Es geht also daraus hervor, dass die Blutmaltase bei Bombyx mori an der Malzzuckers aus der Nahrungsstärke sowie aus dem Körperglykogen Anteil nimmt.

Zusammenfassung.

(1) Im Blute von Bombyx mori kommt eine wirksame Saccharase vor, deren Wirkungsoptimum bei Ph 6.6 liegt.

(2) Die Saccharase des Blutes ist bei gesunden Raupen recht viel starker als bei kranken (Nankabyosan) und auch bei schlecht gewachsenen Raupen etwas höher als bei gut gewachsenen. Durch Hunger ist sie vermindert.

(3) Die Blutsaccharase ist im fünften Lebensalter sehr aktiv, aber dann sinkt sie in den Puppen- und Schmetterlingsperioden allmählich. Die Saccharase des Weibchens ist immer stärker als die des Männchens. Es besteht die folgende Reihenfolge für die Wirksamkeit der Blutsaccharase: Japan-110-E > Europa-7-C > China-7-B.

(4) Die Blutmaltase von Bombyx mori wirkt am besten bei Ph 6.6.

(5) Die Maltasewirkung ist bei kranken Raupen (Nankabyosan) etwas höher als bei gesunden und auch bei schlecht gewachsenen Raupen ein wenig stärker als gut gewachsenen. Durch Hunger verändert sie sich nicht wesentlich.

(6) Im Laufe der drei Entwicklungsperioden ist die Blutmaltase in den Kokonspinnens am aktivesten, wird während der Puppenperiode allmählich schwächer und ist in den Tagen des Schmetterlingslebens wieder bedeutend stärker. Die Maltase des Weibchens ist stets aktiver als die des Männchens. Es besteht kein wesentlicher Unterschied in der Maltasewirksamkeit der drei Rassen.

(7) Aus den oben erwähnten und anderen Tatsachen lässt sich schliessen, dass diese beiden Blutdisaccharasen bei Bombyx mori für die Verdauung bezw. den Stoffwechsel eine wichtige Rolle spielen.

Literatur.

(1) K. Yamafuji: Bull. Agr. Chem. Soc. Japan, **9**, 172, (1933); **10**, 15, (1934).
(2) Derselbe: Ebendaselbst, **8**, 156. (1932); **9**, 172, (1933).

Synthesis of 5-Oxy-camphor.

By

Kunijiro Takeuchi.

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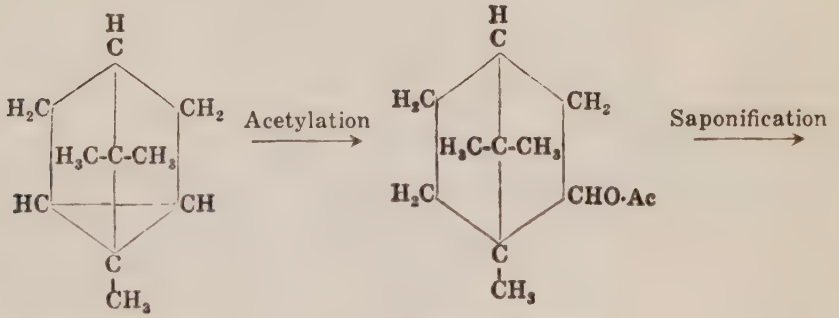
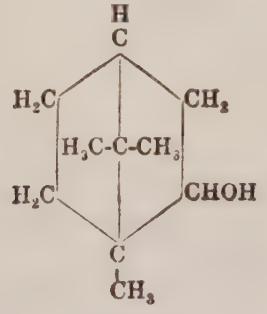
According to Y. Asahina and M. Ishidate[1] 5-oxy-camphor is a chief component of "Campherol"[2] which is obtained from the urine of dogs administered with camphor. The present author has tried to prepare it synthetically.

L. Ruzicka and Liebi obtained isoborneol[3] according to Bertram and Walbaum's method by acetylating α-pericyclocamphane with glacial acetic acid and sulphuric acid and then saponifying the acetylated product as shown in the following scheme:



α-Pericyclocamphane Acetyl-isoborneol

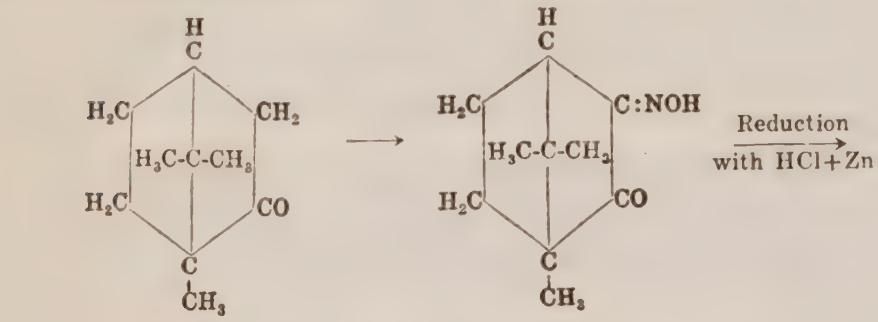


Isoborneol

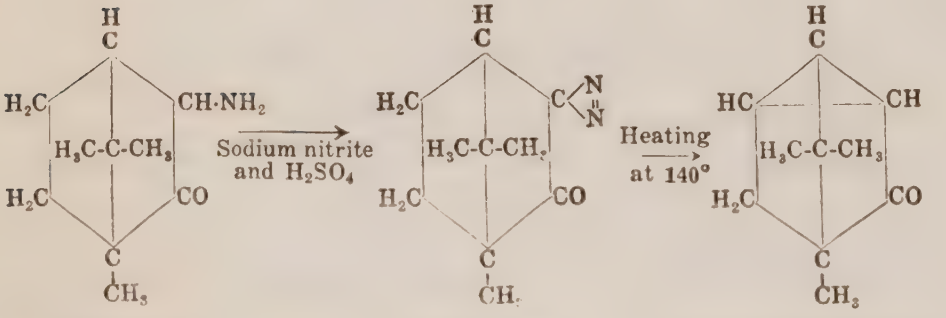
Applying this method to β-pericyclocamphanone, and using trichloracetic acid in place of glacial acetic acid, the present author succeeded in synthesizing 5-oxy-camphor which was identical with the one isolated from dog's urine

according to the method of Asahina and co-workers. The starting material, β-pericyclocamphanone, was prepared by heating diazocamphor at 140°C. There are two methods for the preparation of diazocamphor: one is amino camphor-method,[4] and the other is camphorhydrazone-method.[5] These methods are briefly stated in the following schemes.

[A] Aminocamphor-method.

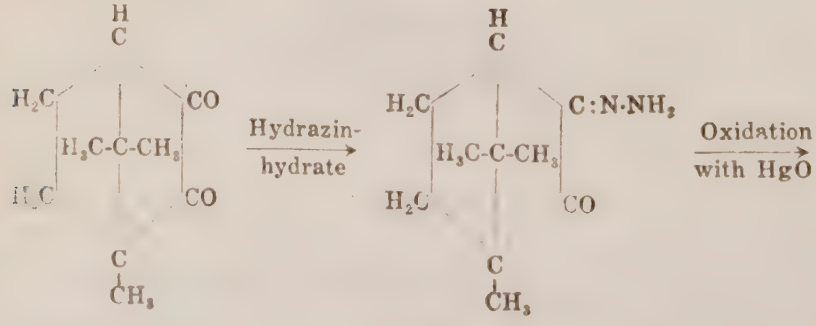


Camphor — Isonitrosocamphor

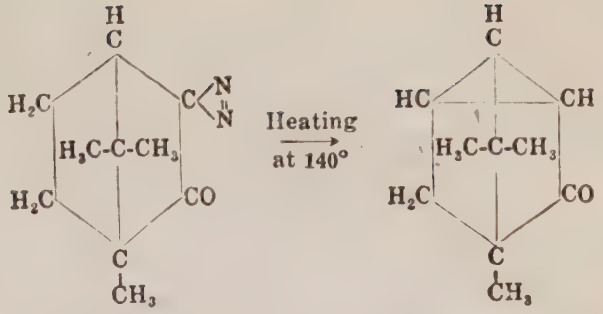


Aminocamphor — Diazocamphor — β-Pericyclocamphanone

[B] Camphorhydrazone method.

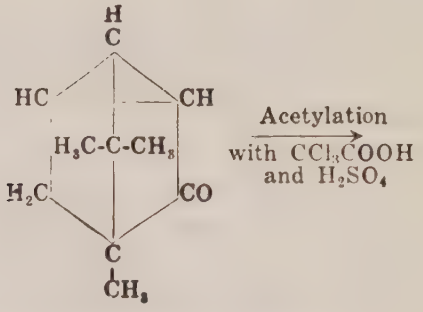


Camphorquinone — Camphorquinone-monohydrazone

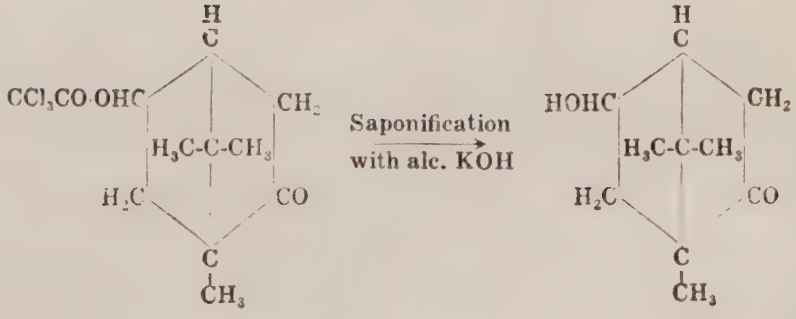


Diazocamphor β-Pericyclocamphanone

5-Oxy-camphor from β-pericyclocamphanone was prepared as follows:

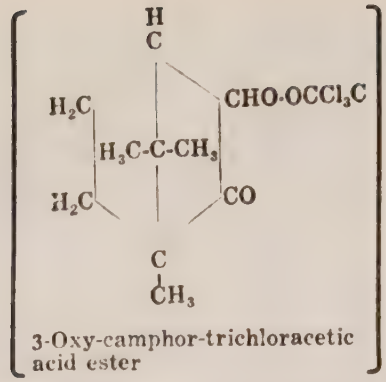


β-Pericyclocamphanone



5-Oxy-camphor-trichloracetic acid ester

5-Oxy-camphor



3-Oxy-camphor-trichloracetic acid ester

Experimental.

Part I. *Preparation of β-Pericyclocamphanone.*

A. Aminocamphor-method.

(1) Isonitrosocamphor.

Five hundred grams of commercial camphor (m. p. = 176~7°C, $[\alpha]_D^{15} = +46.99°$ in abs. alc. c = 7.08) was dissolved in 1250 c.c. dry ether in a flask, to which 74 g metallic sodium was added. Cooling the flask with ice at 10~15°C, dry methyl nitrite gas (which was generated by dropping a solution of 230 g sodium nitrite in 500 c.c. water into the mixture of 400 c.c. water, 120 c.c. methyl alcohol, and 90 c.c. concentrated sulphuric acid) was passed into the flask. After several hours, the etherial solution was shaken with water and the aqueous solution of the sodium isonitrosocamphor was washed with ether to remove borneol and the excess of camphor, and then neutralized with dilute hydrochloric acid. Isonitrosocamphor thus obtained was recrystallized from dilute methyl alcohol; prismatic crystals; yield: 167 g.

m. p. = 151~152°C (uncorr.)
$[\alpha]_D^{24} = +200°$ in abs. alc. ($c = 3.90$)

(2) Aminocamphor sulphate.

One hundred grams of isonitrosocamphor was dissolved in 1,000 c.c. 50% hydrochloric acid and reduced with 200 g zinc dust. Sufficient sodium hydroxide solution was added and the oily aminocamphor separated thereby was then dissolved in ether and added with dilute sulphuric acid drop by drop until the aqueous solution became slightly acid. On evaporating the solution, aminocamphor sulphate separated out as white lustrous crystals. Yield: about 65 g;

m. p. = 213~214°C (uncorr.)
$[\alpha]_D^{15} = +9.51°$ in water ($c = 4.51$)

(3) Diazocamphor.

By adding sodium nitrite and dilute sulphuric acid into the aqueous solution of aminocamphor sulphate, cooling by means of ice, diazocamphor separated out as yellow crystals. It was then purified from petroleum ether. Yield: 78 g pure diazocamphor from 210 g aminocamphor sulphate;

m. p. =74~75°C (uncorr.)
Analysis: 4.293 mg subs. gave 0.5880 c.c. N_2 (746.5 mm, 22°C)
N=15.57%
Calc. for $C_{10}H_{14}ON_2$ N=15.73%

(4) β-Pericyclocamphanone.

β-Pericyclocamphanone was obtained by heating, a small portion at a time, the mixture of 10 g finely powdered crystals of diazocamphor and 30 g dried copper powder in a retort at 140°C. It was purified by sublimation and subsequently recrystallized from petroleum ether. Yield: 8.5 g;

m. p. =167°C (uncorr.)
$[\alpha]_D^{18} = +63.35°$ in abs. alc. (c=1.61)
Analysis: 3.710 mg subs. gave 10.785 mg CO_2, 3.120 mg H_2O;
C=79.28% H=9.40%
Calc. for $C_{10}H_{14}O$ C=79.92% H=9.42%

B. Camphorhydrazone-method.

(1) Camphormonohydrazone.

Twelve grams of hydrazine hydrate was gradually added, small portion at a time, into the boiling alcoholic (40 c.c.) solution of camphorquinone (25 g) (Kahlbaum's preparation, m. p. =195~196°C, $[\alpha]_D^{20} -80.82°$ in benzol, c=1.69). The hydrazone separated on cooling was recrystallized from alcohol. Yield: 20.3 g;

m. p. =198~199°C (uncorr.)
Analysis: 4.716 mg subs. gave 0.6331 c.c. N_2 (762 mm, 19°C)
N=15.73%
Calc. for $C_{10}H_{16}ON_2$ N=15.55%

(2) Diazocamphor.

Thirteen and eight-tenths grams of camphormonohydrazone was dissolved in 100 c.c. hot benzol and then added 22 g yellow mercuric oxide, and the mixture was heated on a water bath for ten hours. Diazocamphor thus separated out was purified from petroleum ether. Yield: 10.3 g:

m. p. =72°C (uncorr.)
Analysis: 2.642 mg subs. gave 0.3420 c.c. N_2 (763 mm, 13.2°C)
N=15.50%
Calc. for $C_{10}H_{14}ON_2$ N=15.73%

(3) β-Pericyclocamphanone.

β-Pericyclocamphanone was prepared from the diazocamphor in the same manner as described in the previous experiment [A]. (4);

m. p. = 167°C

$[\alpha]_D^{17} = +63.37°$ in abs. alc. ($c = 3.66$)

Analysis: 2.849 mg subs. gave 8.240 mg CO_2, 2.357 mg H_2O;
C = 78.9% H = 9.26%
Calc. for $C_{10}C_{14}O$ C = 79.92% H = 9.42%

Part II. *Preparation of 5-Oxy-camphor.*

(1) Acetylation product of β-pericyclocamphanone.

Five grams of β-pericyclocamphanone, 20 g trichloracetic acid and 0.5 g concentrated sulphuric acid were mixed in a flask provided with air-cooler, and heated in oil-bath at 150~160°C for ten hours. After cooling, the resinous mass was extracted with ether and the etherial solution was successively washed with water, with 5% sodium carbonate solution, and again with water. After evaporating the ether off, the residue was subjected to steam distillation in order to remove the excess of β-pericyclocamphanone. The residue was then extracted with ether, and the etherial solution was treated with animal charcoal, and succesively dehydrated, evaporated and distilled. The distillate thus obtained became pasty on keeping in ice-box for several days. Yield: 1 g;

b. p. = 4 mm 140~145°C (uncorr.)

$[\alpha]_D^{17} = +9.30°$ in abs. alc. ($c = 3.44$)

Analysis: 3.606 mg subs. gave 6.270 mg CO_2, 1.736 mg H_2O;
C = 47.42% H = 5.3%
Calc. for $C_{12}H_{15}O_3Cl_3$ C = 45.93% H = 4.7%
10.34 mg subs. gave 12.43 mg AgCl;
9.97 " " " 12.16 " "
Cl = 29.74%
" = 30.17%
Calc. for $C_{12}H_{15}O_3Cl_3$ Cl = 33.9%

Trichloracetic acid ester of 5-oxy-camphor from dog's urine was prepared by the same way as mentioned above.

One half gram f 5-oxy-camphor and 1 g trichloracetic acid were placed in a flask with air-cooler, and heated in oil-bath at 150°C for two hours. The reaction product was then subjected to steam distillation and the residue was extracted with ether, washed with animal charcoal. After dehydration and evaporation, it was kept in a vacuum desiccator for several days, and analyzed as follows;

$[\alpha]_D^{16} = +26.60°$ in abs. alc. ($c = 1.84$)

Analysis: 10.18 mg subs. gave 12.38 mg AgCl
10.06 " " " 12.22 " "
Cl = 30.09%
" = 30.03%
Calc. for $C_{12}H_{15}O_3Cl_3$ Cl = 33.39%

(2) 5-Oxy-camphor.

Two grams of acetylation product of β-pericyclocamphanone mentioned

above were dissolved in 94% alcohol containing 5 g potassium hydroxide, and heated in a flask, fitted with reverted cooler, on water-bath for two hours. After cooling, the mixture was neutralized by passing CO_2 gas and then distilled to remove the alcohol. The residue was then extracted with ether, and after washing the etherial solution with water, it was dehydrated and evaporated. On recrystallization from hot ligroin (b. p.=80~120°C), it formed nice characteristic crystals (Photo. 2) which were colourless, odourless, and adhesive as camphor; hardly soluble in cold ligroin, but easily soluble in alcohol, ether, benzol and acetic acid. Yield: 0.5 g;

m. p.=222~222.5°C (uncorr.)
$[\alpha]_D^{17}=+42.7°$ in abs. alc. ($c=0.34$)
$[\alpha]_D^{18}=+43.5°$ in abs. alc. ($c=2.09$)

Mixed with the specimen of 5-oxy-camphor (m. p.=222°C, $[\alpha]_D^{14}=+43.9°$, Photo. 1) prepared from dog's urine, no depression of melting point was observed.

Molecular weight (freezing point depression method);

0.1286 g subs. in 33.3 g benzol $\Delta=0.11°$ mol. wt.=170.5
Calc. for $C_{10}H_{16}O_2$.....mol. wt.=168
Analysis: 3.440 g subs. gave 8.989 mg CO_2, 2.934 mg H_2O;
C=71.27% H=9.54%
Calc. for $C_{10}H_{16}O_2$ C=71.4% H=9.5%

Semicarbazone of the above synthetic 5-oxy-camphor was prepared in the usual way;

m. p.=232~3°C
Analysis: 3.979 mg subs. gave 0.6497 c.c. N_2 (753 mm, 15.0°C)
N=19.18%
Calc. for $C_{11}H_{19}O_2N_3$ N=18.6%

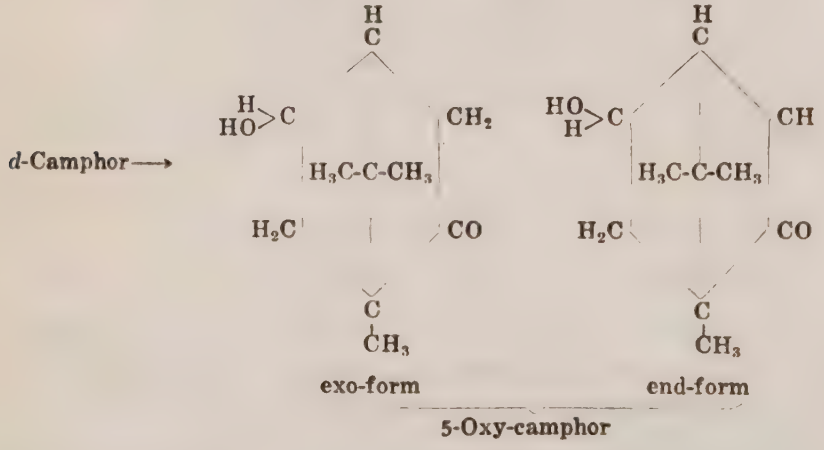
Acetyl-5-oxy-camphor was prepared by heating 1 g of the above 5-oxy-camphor and 1 c.c. acetic anhydride on oil-bath at 140°C for two hours. The excess of acetic anhydride was decomposed with water, and the reaction product was extracted with ether. The etherial solution was washed with 5% sodium carbonate solution, evaporated and distilled;

b. p.=23 mm 145~147°C (uncorr.)
$[\alpha]_D^{19}=+26.27$ in abs. alc. ($c=1.18$)
Analysis: 4.079 mg subs. gave 10.105 mg CO_2, 3.036 mg H_2O;
C=67.57% H=8.33%
Calc. for $C_{12}H_{18}O_3$ C=68.6% H=8.6%

The preparation of synthetic 5-oxy-camphor agreed with that obtained from dog's urine by Y. Asahina and M. Ishidate as shown in the table below:

	Y. Asahina and M. Ishidate	The present author
5-Oxy-camphor	m. p. = 222° (217~8°)C $[\alpha]_D^{20} = +43.2°(41.3°)$ in alc. C=71.04% H=9.6%	m. p. = 222~222.5°C $[\alpha]_D^{18.0} = +42.7\sim43.5°$ in alc. C=71.27% H=9.54%
5-Oxy camphor-semicarbazone	m. p. = 233~5°C N=19.1%	m. p. = 232~3°C N=19.1%
Acetyl-5-oxy-camphor	b.p. = 27 mm 159~60°C $[\alpha]_D^{20} = +24.8$ in alc. C=68.58% H=9.00%	b. p. = 23 mm 145~7°C $[\alpha]_D^{19} = +26.27$ in alc. C=67.57% H=8.33%

After all, starting with *d*-camphor, the author has succeded in synthesizing 5-oxy-camphor which was identical both in physical and chemical properties with the preparation isolated from dog's urine. Theoretically, however, two stereoisomers of 5-oxy-camphor can be produced from *d*-camphor as shown in the following scheme:



Further investigations on this subject are still being carried on and the reports will be published in near future.

The author whishes to express his sincere thanks to Professors U. Suzuki and T. Yabuta for their kind guidances throughout this work, and also to Dr. Y. Sahashi for his kihd advices. For analyses, the author is indebted to Mr. S. Funahashi and Miss S. Matsumoto.

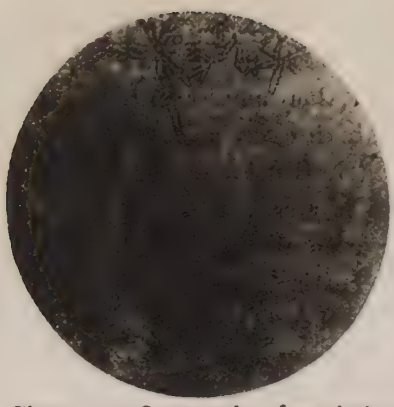


Photo. 1—5-Oxy-camphor from dog's urine.

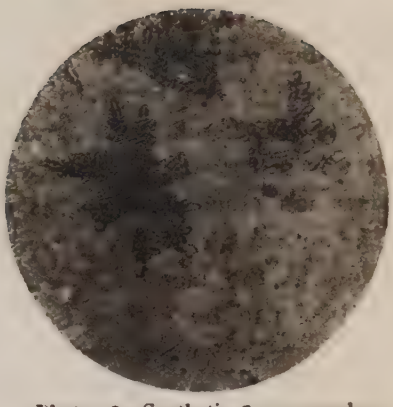


Photo. 2—Synthetic 5-oxy-camphor.

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Studies on Amylosynthease.

By

Toyosaku MINAGAWA.

(Agricultural Chemical Laboratory, Tokyo Imperial University, Komaba, Tokyo.)

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I. Introduction.

The existence of an enzyme in the autolysed extract of brewer's yeast which has the power of polymerising achroodextrines into higher ones was first observed by S. Nishimura[1] in 1930 and the name "Amylosynthease" was given to it. The present author continued the studies on this subject, at first in association with the above named author and afterwards independently and succeeded in preparing the enzyme quite free from amylase, which occurs always associated with the former and interferes the polymerisation process, exerting the reverse reaction.

From the studies on the chemical nature of this enzyme, behaviors towards various reagents, optimum temperature and pH value for its activities etc., the author has come to the conclusion that amylosynthease is a new enzyme quite different from amylase and the both exert no reversible reaction.

It was observed that this enzyme is widely distributed in many varieties of yeasts and other microbes, as well as in higher plants.

The author has further proved that trihexosan and hexahexosan, prepared according to the method of A. Pictet, by heating starch with glycerine at 200～210°C can be polymerised by amylosynthease and has isolated hexahexosan from the polymerisation products of trihexosan. This gives strong evidence that the enzyme has synthetic power.

It was further observed that the dextrines prepared by the action of various amylolytic enzymes upon starch can be generally polymerised to the higher ones giving blue colouration with iodine, while the lower dextrines obtained by the action of mineral acids upon starch can never be regenerated though they seem to be identical with the products obtained by enzymes. This provides a new means of distinguishing the dextrines prepared by different methods.

The author has also determined the physico-chemical constants of polymerisation products or the socalled "synthetic starches", such as viscosity, saponification value, acetyl value, rotatory power etc. and found close resemblance with those of natural soluble starches. The X-Ray spectrogram and photometric curves have also great similarity with natural ones. But, at present, there is no means of settling the question whether the synthetic products are really identical with natural ones.

Finally, the author has demonstrated the existence of a complement of amylosynthease in the yeast extract.

In the following is given a brief summary of the results reported by the author in the Journal of Agricultural Chemical Society of Japan[2] and also in the proceedings of the Imperial Academy of Japan.[3]

II. Isolation of amylosynthease.

(1) Preparation of yeast extract.

Fresh beer yeast was well washed with water, pressed between the filter papers and dried in a vacuum desiccator. 10 g of the dried yeast thus obtained were introduced into the flask, with 100 c.c. water. After adding 10 c.c. toluol the flask was vigorously shaken and kept for two days at 25～26°C. The extract was now filtered through paper and the clear filtrate itself was used for the experiment.

(2) Soluble starch solution: Soluble starch was prepared by treating

the starch of glutinous rice with cold 7.5% hydrochloric acid solution. After removing the acid by suction, it was extracted with boiling water and filtered. The filtrate thus obtained gave a red colour with iodine and could be used directly for the experiment. In some cases, however, the soluble starch was isolated from the above filtrate by precipitating with strong alcohol.

(3) The polymerisation by amylosynthease was demonstrated in the following experiment. 2 liters of a 1% clear solution of soluble starch (pH 6.2) were introduced in a large erlenmeyer flask with 100 c.c. of the yeast extract prepared as above mentioned. After adding 7 drops of chloroform to prevent the bacterial growth, the flask was stoppered with cork plug and kept in a thermostat at 25~26°C. The clear solution became soon opaculent and a white flocculent precipitate was formed, which gradually grew larger until at last a very voluminous mass of the precipitate separated out.

The original solution, giving a red colour with iodine, became after 6 hours violet in colour, which turned into bluish violet more and more as the time went on. After standing for a week, the precipitate was collected on a filter, washed with alcohol and ether, and dried. The yield was about 84 % of the original soluble starch used. The white amorphous powder thus obtained dissolved in hot water almost completely, but precipitated out again on cooling. It gives a bluish violet colour with iodine.

(4) The control soluble starch solution, without any addition of the yeast extract, but otherwise treated in the same way as above, remained unchanged for a month and not even any slight turbidity was produced in it and when treated with iodine, the solution gave only a red colour.

(5) Isolation of amylosynthease from yeast extract.

The clear yeast extract was saturated with ammonium sulphate, whereby a flocculent precipitate was produced, which carried down a greater part of amylosynthease. This precipitate was collected on a filter and freed from the mother liquid by suction, washed with a little cold water to remove the adhering ammonium sulphate, washed again with ether and dried in a vacuum desiccator.

The yellowish white powder thus obtained was still contaminated with a little amylase. Now the author has observed that amylase is easily soluble in glycerine, while amylosynthease is not. Thus, by washing the above preparation thoroughly with glycerine, the amylase was completely removed. The amylosynthease thus prepared was found to be very active.

(6) Fractional precipitation of amylase and amylosynthease.

When 100 c.c. of yeast extract was treated with 28~30 g ammonium sulphate, a flocculent precipitate was formed, which carried down a greater part of amylase.

The filtrate therefrom gave when saturated with ammonium sulphate, a second precipitate retaining chiefly amylosynthease, which may be further purified by washing with glycerine.

The separation of the two enzymes was thus easily effected.

The glycerine extract of the first precipitate had strong amylolytic power and acted on the boiled potato starch very quickly until the solution gave no colouration with iodine.

(7) Precipitation of amylosynthease by lead acetate.

When lead acetate was added to the yeast extract until it reached about 1% of the solution, a white flocculent precipitate was formed, including a greater part of amylosynthease and leaving amylase in solution.

This precipitate was now collected on a filter by suction and after washing with cold water, it was suspended in water and treated with a small quantity of sodium bicarbonate, while passing CO_2 gas in it, whereby the amylosynthease was dissolved out in the solution and the filtrate therefrom exhibited a strong polymerising power upon soluble starch.

(8) Isolation of amylosynthease from potato tubers.

Fresh potato was crushed to a fine pulp, adding the same volume of a 1/30 mol. phosphate buffer solution (pH 8.0), filtered through cloth and then through paper. The filtrate thus obtained was now saturated with ammonium sulphate, whereby a flocculent precipitate was formed, including both amylase and amylosynthease. By thoroughly washing the precipitate with glycerine, however, the amylase could be removed completely and the insoluble residue retained only amylosynthease.

(9) Isolation of amylosynthease from polished glutinous rice.

Polished glutinous rice is finely powdered, macerated with 3~4 volumes of cold water, filtered through cloth and then through paper. When the filtrate thus obtained is treated with 1/100~1/160 mol. solution of cadmium chloride, a yellowish white precipitate is formed, which carries down amylosynthease leaving amylase in solution. The precipitate is now suspended in water and saturated with ammonium sulphate, whereby an active amylosynthease is obtained.

III. General properties of amylosynthease.

(1) Elementary composition of amylosynthease.

The amylosynthease precipitated by cadmium chloride from the extract of beer yeast, or baker's yeast (Fleischmann's yeast) or from the extract of glutinous rice and purified by treating with ammonium sulphate, following by washing with glycerine is a white amorphous powder, easily soluble in cold water and gives biuret, xanthoprotein and Molisch's reaction.

They contain in average 8% nitrogen, 30% carbon, 5% hydrogen, 28% cadmium chloride and 5% ash.

The existence of a little sulphur and phosphorous is also detected.

(2) Behaviours toward various reagents.

The amylosynthease is completely precipitated from its aqueous solution by adding lead acetate or cadmium chloride and can be regenerated from the precipitate by the methods above mentioned. The amylase is thereby left in solution.

The amylosynthease is also precipitated by a little mercuric chloride, but it can never be regenerated from this precipitate by any treatment, while amylase inactivated by the same reagent can be easily regenerated by treating the precipitate with hydrogen sulphide. Thus, we see that there is a remarkable difference between amylase and amylosynthease in the behavior towards various reagents and the latter must be considered to be a new enzyme quite different from amylase, though it occures always associated with the latter.

Further, amylosynthease is completely thrown down from its aqueous solution by adding a little lead nitrate, ferric nitrate, ferric chloride, cadmium nitrate, aluminium nitrate or aluminium chloride.

When the precipitates, thus obtained are suspended in a little cold water and saturated with ammonium sulphate, white insoluble residues are obtained, which retain an active enzyme.

The amylosynthease is also precipitated by silver nitrate, copper nitrate, copper chloride, copper acetate, mercuric acetate, platinium chloride and zinc chloride. But, it can never be regenerated from these precipitates by any treatment.

Cobalt chloride, nickle chloride, magnesium chloride and barium chloride have no power of precipitating the enzyme from the solution.

The precipitate of amylosynthease formed by the addition of mangan chloride, ammonium alum and feather alum behaves as such like the enzyme itself, when added to the solution of soluble starch.

Thus, we see that the behavior of various metalic salts upon amylosynthease is quite defferent according to the nature of metals.

(3) Protective action against chemical reagents.

Toluol has no inhibiting action, while chloroform behaves noxiously upon amylosynthease. The noxious effect of the latter, however, can be protected by dextrine. Thus, when a solution of dextrine is treated with a little amylosynthease and after adding a few drops of chloroform, stoppered with cork plug, slightly shaken and kept in a thermostat at 20~25°C, the polymerisation proceeds very quickly.

Formaldehyde, carbon disulphide and carbon tetrachloride behave also harmfully like chloroform.

(4) The amylosynthease is precipitated by alcohol and aceton, and at the same time becomes insoluble in water. The precipitate obtained by saturating with ammonium sulphate is easily soluble, but it can not be precipitated again by the same reagent.

(5) Action of pancreatic tryptase on amylosynthease.

When 1～2% aqueous solution of pancreatine (Japanese Pharmacopoea) is added with ammonium sulphate up to 24～25%, a white flocculent precipitate is obtaind. This precipitate is collected on a filter, dissolved in 30 times of water after drying in vacuum and treated with aceton up to 80%, whereby tryptase is again precipitated. By repeating this process, tryptase quite free from amylase can be obtained.

This pancreatic tryptase has no action upon amylosynthease, so it is presumed that amylosynthease is not of protein nature, though it is precipitated by ammonium sulphate in its impure state.

(6) Adsorption of amylosynthease.

The amylosynthease is adsorbed by aluminium hydroxyde, aluminium phosphate, monocalcium phosphate, caolin and animal charcoal. From these adsorbants, the enzyme can be again dissolved out by treating with a dilute sodium bicarbonate solution.

Amylase is not adsorbed by caolin, so it can be easily separated from amylosynthease.

(7) Optimum temperature and opt. pH value.

The amylosynthease is very sensitive to high temperature, when the yeast extract is preserved at 30°C, the activity is nearly lost in a day, while it is quite stable at lower temperature (0～15°C) and may be kept for a month without loosing much in activity. In dry state, however it can be preserved at room temperature for several months.

The opt. temperature is estimated to be 20～25°C; it is completely destroyed by heating at 50°C for 10 minutes.

The opt. pH value of yeast amylosynthease is 6.2 and that of potato and rice is 6.8～7.0.

IV. Distribution of amylosynthease.

Amylosynthease seems to be widely distributed in various kinds of yeasts, thus it is detected in beer-, wine-, distiller's and baker's yeasts, as well as in saccharomyces ellepsoideus, s. biwa, s. eryobotoryae, s. apiculatus, film forming yeast and mycoderma; but, it is absent in saccharomyces saké, s. pasteurianus, zigos. major, willia anomala, pichia menbranaceae, tolura A.,

oidium lactis, botrytis, aspergilus oryzae, bac. subtilus, bac. coli, and acetic bacteria.

Its presence is also confirmed in potato, common and glutinous rice.

V. Polymerisation of trihexosan and hexahexosan.

The author has prepared trihexosan and hexahexosan according to the method of A. Pictet by heating starch with glycerine at 200~210°C and confirmed that these two compounds can be polymerised into higher ones by the action of amylosynthease, thus, the aqueous solution of trihexosan which gave no colouration with iodine, become after adding a little amylosynthease, red, violet and finally bluish violet. Hexahexosan which gives red colour with iodine behaved also in the same manner.

The author has further isolated hexahexosan from the polymerisation products of trihexosan. For this purpose, the aqueous solution of trihexosan was treated with a little amylosynthease and when the iodine reaction became red, the solution was evaporated and poured into a large quantity of alcohol and the precipitate thus formed was subjected to fractional precipitation with alcohol. In this way, hexahexosan was isolated and was proved to be identical with the product obtained by the method of A. Pictet.

VI. Polymerisation of glycogen and dextrine prepared from different origins by different methods.

(1) Polymerisation of glycogen.

The glycogen used in this experiment was prepared by digesting commercialy purified glycogen with cold 7.5% hydrochloric acid and precipitating with alcohol after 2 or 3 days. It dissolved clearly in water and gave a brownish red colour with iodine. Now, by adding a little amylosynthease to the above solution, the polymerisation proceeded at once and the colour gradually changed to red, violet and finally to blue.

It is interesting to note that genuine glycogen which can scarcely be acted by amylosynthease, becomes easily polymerisable after treating with cold hydrochloric acid as above stated.

The degradation products of glycogen, obtained by the action of amylase can also be polymerised.

(2) Polymerisation of dextrines contained in various grains.

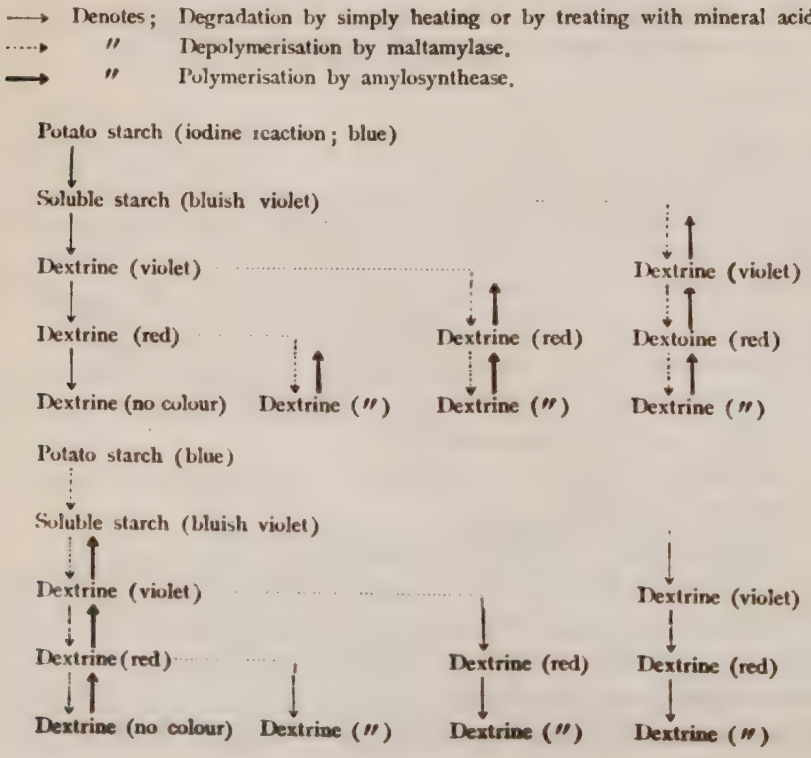
Natural dextrines seem to be widely distributed in various kinds of grains. For the preparation of it, finely powdered grain is boiled with 95% alcohol and the insoluble residue is extracted with cold water. The dextrines thus obtained give generally red or brown colour with iodine, but sometimes the reaction is negative. All these preparations are proved to be

polymerisable by amylosynthease. Soluble starches prepared from glutinous millet (*Panicum miliaceum*) and foxtail millet (*Setalica italica*) by treating with cold 7.5% hydrochloric acid are also polymerised by amylosynthease and form insoluble precipitates with closely resemble to the products obtained from rice dextrine. Further, the dextrines and soluble starches were acetylated and purified by decolorizing with animal charcoal and by precipitating again with alcohol. When the acetylated products thus obtained were saponified with alcoholic potash to remove acetyl groups and the regenerated dextrines were treated with amylosynthease the polymerisation proceeded at the same rate as before acetylation. It was observed, however that the dextrines, except "grenzdextrine", have undergone certain changes, because the degradation products of them could never be polymerised again by amylosynthease. Similarly the degradation products obtained by the action of mineral acids upon dextrines are not polymerisable.

(3) Polymerisation of dextrines prepared by various methods.

It was observed that the dextrine prepared from potato starch by maltamylase can be easily polymerised by amylosynthease, while those prepared by simply heating or by treating with mineral acids can never be polymerised. It seems thus these dextrines are chemically different, though the both give red coloration with iodine.

Further, various kinds of dextrines were prepared and their behavior toward amlosynthease was observed with the following results:



It can be seen that in the above experiments, chemically different dextrines are always produced according to the method of preparation.

The author has prepared dextrines, which give no colouration with iodine, i. e. β-glucosidomaltose (R. Ling) and amylotriose (H. Pringsheim) by the methods above mentioned and found that it was impossible to distinguish them by chemical or physical means, but the behavior toward amylosynthease was quite different, i. e. β-glucosidomaltose could be polymerised, while amylotriose not.

As the reducing power of dextrine disappears after polymerisation, it is presumed that free aldehyde group is responsible for the polymerisation and in the case of trihexosan and hexahexosan, free aldehyde group might be formed instanteniously in the molecule during polymerisation.

VII. Relative velocity of polymerisation of different dextrines.

With the purpose of determining the relative velocity of polymerisation of different dextrines, each 1% solution of soluble starch and amylose of glutinous rice, achroodextrine, erythrodextrine and hexahexosan was prepared and treated with the amylosynthease solution (prepared by macerating 150 g dry yeast with 900 c.c. water) at room temperature and the times required until the solution give bluish violet colour with iodine were estimated. The results are graphically shown in Fig. 1.

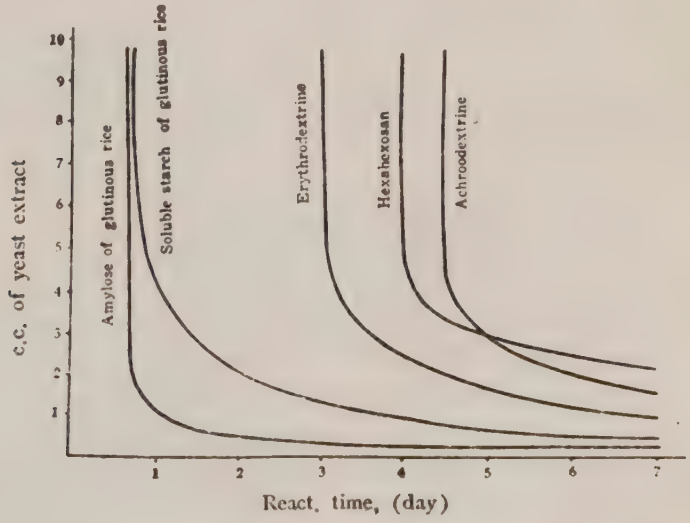


Fig. 1. 25 c.c. of 1% solution (hexahexosan, achroodextrine, erythrodextrine, amylose or soluble starch of glutinous rice) is added with yeast extract (at room temperature).

From these results, it can be seen that the dextrines of larger molecules can be more quickly polymerised than those of smaller molecules.

In Fig. 2 the activity of amylosynthease previously kept in aqueous solution at different temperature is graphically described.

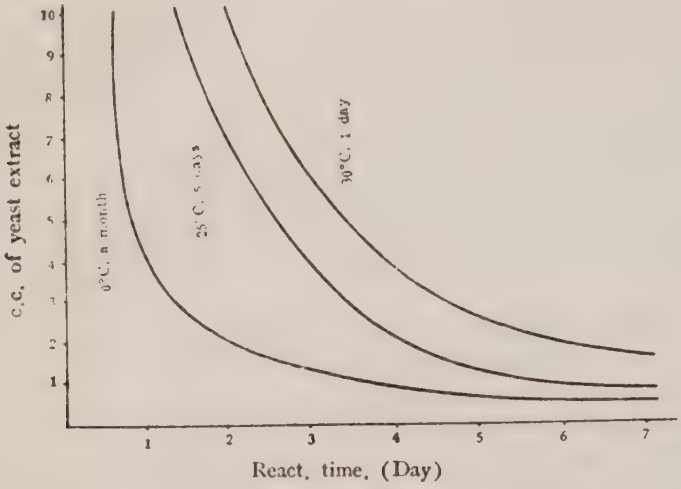
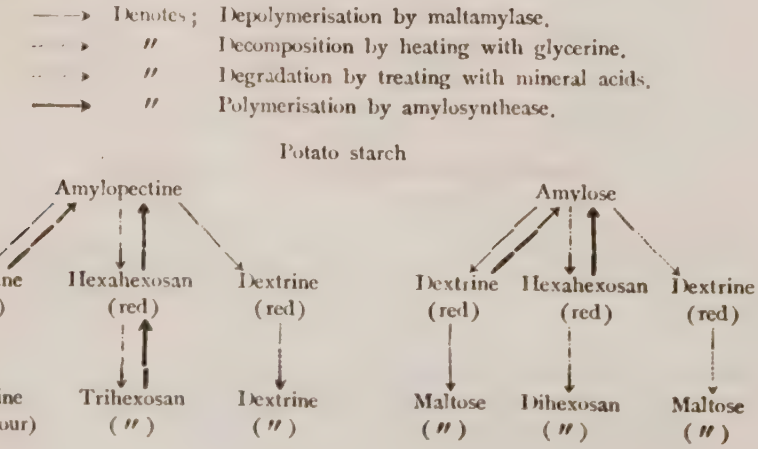


Fig. 2. The yeast extracts previously kept for 1 day at 30°C, for 5 days at 25°C and for a month at 0°C resp., were added each to 25 c.c. of 1% solution of soluble starch of glutinous rice. (at roomtemperature)

VIII. Polymerisation of the derivatives of amylose and amylopectine.

(1) Amylose and amylopectine were treated either by maltamylase, by heating with glycerine or by treating with conc. hydrochloric acid and the behaviors of these decomposition products toward amylosynthease were observed with the following results:



By such a means, it is possible to determine whether the chemical configuration of certain carbohydrates are analogous to amylose or to amylopectine.

(2) On lichen starch (Isolichenin).

Isolichenin is widely distributed in many varieties of japanese mooses, i. e. in alectoria ochrolenca, pertusalia trocophota, ramalina genuculata, spharophorus collaloidrs, thamnolia vermicularis, usnea trichodea, cetralia islandica, etc..

It forms a white amorphous powder, clearly soluble in hot water and gives blue coloration with iodine. It is interesting to note that isolichenin (from alectoria ochrolenca) is gradually decomposed by maltamylase giving blue, violet, red and finally yellow coloration with iodine just like starch.

The behaviors of these decomposition products toward amylosynthease were compared with those of glycogen and it was confirmed that the configuration of isolichenin is analogous to amylose and that of glycogen to amylopectine.

IX. Physico-chemical properties of synthetic starches.

4 kinds of synthetic starches were prepared by amylosynthease from different sources, i. e, from soluble starches of glutinous rice and millets (A), from dextrine prepared by maltamylase (B), from hexahexosan (C) and from soluble glycogen (D), and their general composition, saponification value, viscosity, rotatory power as well as the acetyl value were estimated.

It was confirmed that these synthetic products have great similarity with the natural soluble starches.

Further, it was shown that the preparation (B) and (D) give typical crystalline X-Ray spectrogram and photometric curves which closely resemble to those of natural starches (Fig. 4).

X. On the complement of amylosynthease.

The yeast extract is treated with 70% alcohol and the precipitate thereby formed is further purified by dissolving in water, filtering through paper and again precipitating with 70% alcohol. In this way, a white powder is obtained which is easily soluble in cold water and posseses the power of accelerating the activity of amylosynthease, as is shown in Fig. 3.

When the filtrate of the above precipitate is concentrated in vacuum and treated with 95% alcohol, an yellowish white precipitate is obtained, which has strong accelerating power upon amylase.

Further, it was observed that glutathion and cystein which strongly accelerate amylase have no action upon amylosynthease as shown in the

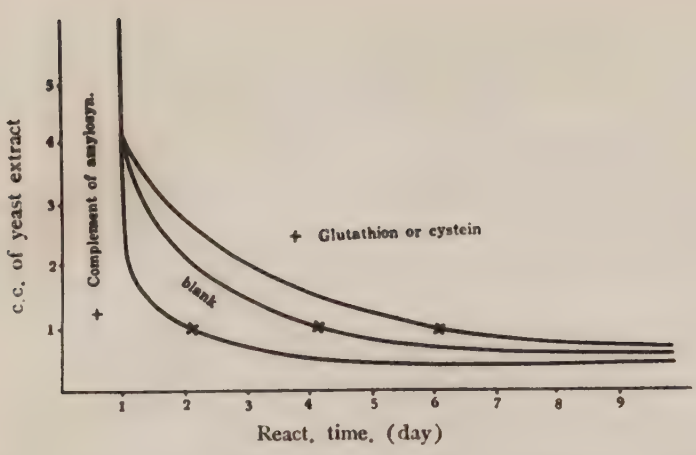


Fig. 3. 25 c.c. of 1% solution of soluble starch of glutinous rice is added with yeast extract, complement of amylosynthease + yeast extract or glutathion or cystein + yeast extract.

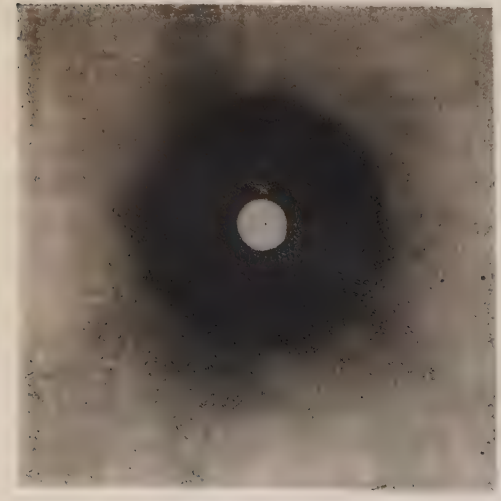
following experiment:

Soluble starch from glutinous rice treated with:	Iodine reaction after:	
	1 day	2 days
Amylosynthease from glutinous rice	red violet	violet
" + Complement	violet	bluish violet
" + glutathion or cystein	brown	yellow

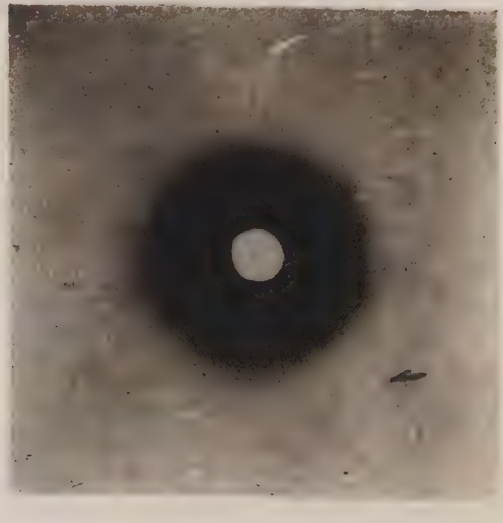
The above result shows that glutathion or cystein exerted rather inhibiting action upon amylosynthease.

Fig. 4. Diffraction figure

No. 1 (Soluble starch)

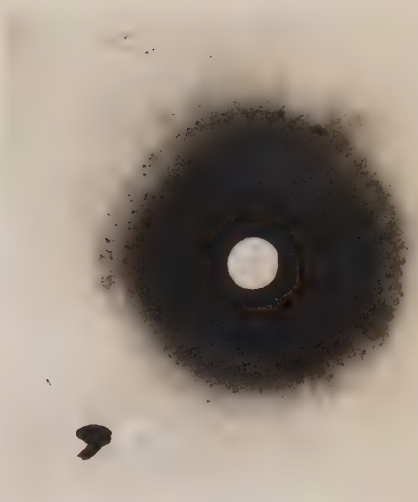


No. 2 (Synthetic starch B.)



No. 3 (Potato starch)

No. 4 (Synthetic starch D.)



Photometric curve

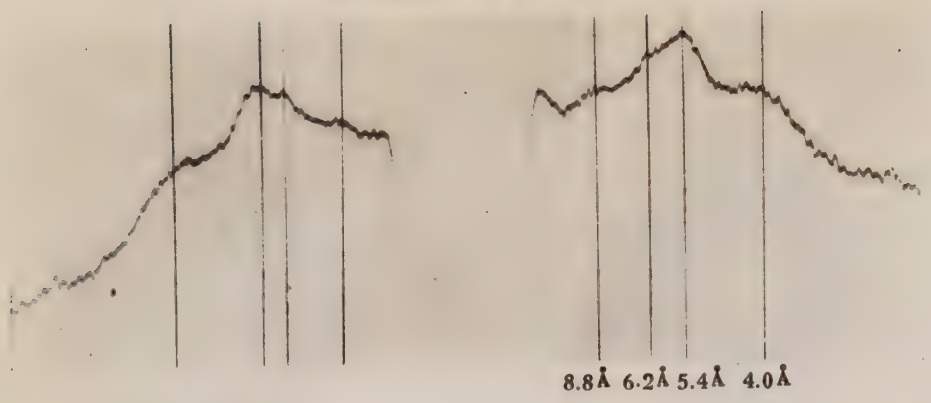
No. 1 (Soluble starch)

55 Å 4.0 Å

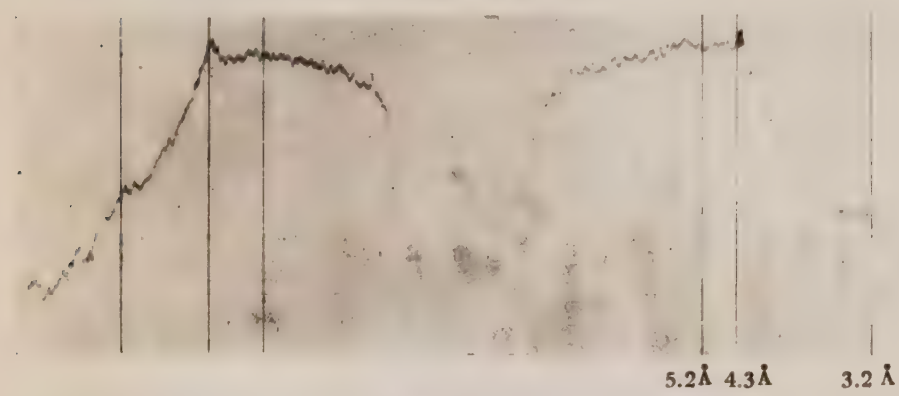
No. 2 (Synthetic starch B.)

7.1 Å 4.6 Å

No. 3 (Potato starch)



No. 4 (Synthetic starch D.)



Literature.

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On the Glycogen Content in the Liver of Pigeons and Rats by B_1-Avitaminosis.

By

Hisasi Ariyama.

(*From the Agricultural Chemical Laboratory of Tokio Imperial University, Komaba.*)

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Introduction.

Many authors such as Funk and Schönborn[1], Ogata[2], Collazo[3], Bickel and Collazo[4] etc. have observed that the liver glycogen of pigeons and fowls suffering from vitamin B-deficiency is always less than the normal healthy ones, sometimes being reduced to the minimum. Quite different result was, however, obtained by Abderhalden and Wertheimer[5], who fed pigeons with polished rice or with synthetic B-free diets and observed an enormous increase of glycogen in the liver and heart of the diseased animals but by giving dried yeast or B_1-preparation, the glycogen content is soon reduced to the normal level. It seems thus that B_1 plays an important rôle for the metabolism of glycogen in the liver. The present experiment was undertaken to contribute more data for the decision of this problem.

Experimental.

(1) Liver Glycogen of Pigeons fed on Polished Rice.

Pigeons weighing about 300 g were divided into two groups and the 1st. (Control) group, consisting of 9 pigeons, was fed on unpolished rice ad. libitum, each bird being kept in a separate wire cage.

The glycogen content in the liver was estimated according to the Pflüger's method modified by Laurence and McCance[6] with the following results:

Table 1. Liver Glycogen of Pigeons fed on Unpolished Rice (Control).

Pigeon No.	Body weight			Liver glycogen. (%)	No. of days fed.
	At the biginning of feeding. (g)	At the end of feeding. (g)	Increase (+) or decrease (−) in weight. (%)		
1	315	321	+ 1.9	2.01	4
2	326	339	+ 4.0	3.49	4
3	328	331	+ 0.9	3.42	4
4	338	344	+ 1.8	2.82	6
5	328	362	+10.4	4.76	6
6	279	272	− 2.5	2.92	6
7	274	294	+ 7.3	2.78	6
8	277	278	+ 0.4	3.29	8
9	288	287	+ 0.3	3.97	9
Ave.				3.28	

The 2nd. group, consisting of 18 pigeons, was fed on polished rice supplemented daily with 0.06 g McCollum and Simmonds salt mixture and 0.4 g autoclaved yeast as the source of B_2. The symptoms of B_1-deficiency were roughly classified into three types, i. e. spastic-, paralytic-and atrophic forms. At the day or the following, when the definite symptom of polyneuritis was developed, 14 pigeons were killed and the liver glycogen was estimated, while the remaining ones were cured by administering each 4 mg active oryzanin (B_1-preparation of Sankyo and Co.) and after complete recovery, the liver glycogen was estimated.

Table 2. Liver Glycogen of Pigeons fed on Polished Rice.

Pigeon No.	Body weight.			Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
	At the beginning of feeding. (%)	At the appearance of the disease. (%)	Decrease in weight. (%)			
1	229	203	−32.1	6.35	23	Spas.
2	339	205	−39.5	6.25	26	Par.
3	335	213	−36.4	5.08	21	〃
4	292	213	−27.1	4.58	36	Spas.
5	336	222	−33.9	6.77	26	Par.
6	332	210	−36.7	3.75	32	〃
Ave. : Pigeons weighing over 200 g. 5.46						
7	321	175	−45.5	0.86	40	〃
8	330	198	−40.0	+	39	〃
9	298	161	−46.0	+	26	〃
10	270	151	−44.0	2.17	32	〃
11	325	170	−47.7	0.82	32	〃
12	306	188	−38.6	1.17	19	Spas.
13	299	145	−51.5	0	22	Par.
14	351	170	−51.3	0	26	Atr.
Ave. : Pigeons weighing below 200 g. 1.26						
15	307	226	−26.4	3.78	16 (4;−3)	Spas.
16	310	223	−28.1	2.37	23 (11;−4)	〃
17	303	189	−37.6	2.82	20 (9;+3)	Par.
18	316	176	−44.3	2.88	50 (9;−1)	〃
Ave. : Pigeons cured by B_1-preparation. 2.96						

Figures in parenthesis indicate No. of days treated with active oryzanin and the increase or decrease of body weight is denoted with (+) or (−).

It can be seen from the above table that there is a great difference in the glycogen content among the pigeons, though they were equally fed on polished rice, namely, those which developed the disease without loosing much in body weight contained larger quantity of glycogen than those which have heavily lost in body weight due to the loss of apetite at the later period of the experiment and suffered from severe inantion. Thus the ave-

rage content in pigeons weighing over 200 g was 5.46%, while those weighing less than 200 g was only 1.26%. Most probably, the glycogen once deposited in large quantity was again used up during the stage of inanition. The pigeons cured by the administration of B_1-preparation had in average 2.96% glycogen. This is nearly the same with that of the control group fed on unpolished rice, i. e. 3.28%.

(2) Liver Glycogen of Pigeons fed on B_1-Free Synthetic Diet.

Pigeons weighing about 300 g were divided into two groups and the 1st. group, consisting of 17 pigeons, was fed on a synthetic diet, deficient in B_1 but otherwise adequate for growth.

It consisted of: Fish meat protein 15, starch 72, butter fat 10, McCollum and Simmonds salt mixture 3. Fish meat protein and starch were purified by extracting with alcohol and ether. Butter fat was treated with hot water and alcohol. As the source of B_2, 0.4 g autoclaved yeast were given daily. The 2nd. (Control) group was fed on the same diet as above except that the autoclaved yeast was substituted with the same amount of dried yeast. The results were as follows:

Table 3. Liver Glycogen of Pigeons fed on B_1-Free Synthetic Diet.

Pigeon No.	Body weight. At the beginning of feeding. (g)	Body weight. At the appearance of the disease. (g)	Decreare in weight. (%)	Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
1	280	250	−10.7	6.93	29	Spas.
2	260	206	−20.8	6.14	26	〃
3	308	246	−22.1	5.52	27	〃
4	335	255	−23.9	6.81	13	〃
5	336	233	−30.7	4.93	36	Par.
6	328	215	−34.4	6.43	32	Spas.
7	321	200	−37.7	6.55	30	〃
8	329	200	−39.2	5.47	38	〃
Ave.: Pigeons weighing over 200 g.				6.10		
9	277	175	−36.8	3.59	33	Par.
10	283	175	−38.1	0.45	34	〃
11	311	172	−44.7	1.29	29	〃
12	305	176	−45.6	0.90	42	〃
13	316	128	−59.5	1.33	35	Atr.
Ave.: Pigeons weighing below 200 g.				1.51		
14	302	224	−26.1	2.46	23 (5;+ 5)	Spas.
15	285	201	−29.5	2.13	19 (8;+14)	Par.
16	273	211	−22.7	1.63	16 (11;± 0)	Spas.
17	320	227	−29.1	2.50	23 (6;+ 9)	〃
Ave.: Pigeons cured by B_1-preparation.				2.18		

Table 4. Liver Glycogen of Pigeons fed on the Complete Synthetic Diet (Control).

Pigeon No.	Body weight. At the beginning of feeding. (g)	At the end of feeding. (g)	Increase (+) or decrease (−) in weight. (%)	Liver glycogen. (%)	No. of days fed.
1	272	268	−1.5	1.90	9
2	272	272	±0.0	2.36	9
3	300	295	−1.7	1.86	11
4	312	295	−5.4	1.47	16
Ave.				1.90	

The above results agree well with that of the 1st. experiment fed on rice diet, i. e. those pigeons weighing over 200 g had in average 6.10% and those weighing less than 200 g contained only 1.51%, while those cured by B_1 had nearly the same amount as that of control ones, i. e. 2.18% against 1.90%.

(3) Liver Glycogen of Underfed Pigeons.

In this experiment, pigeons were fed on restricted amount of unpolished rice together with 0.4 g dried yeast and when the loss of body weight attained 20%～30%, the glycogen was estimated with the following results:

Table 5. Liver Glycogen of Underfed Pigeons.

Pigeon No.	Body weight. At the beginning of feeding. (g)	At the end of feeding. (g)	Decrease in weight. (%)	Liver glycogen. (%)	No. of days fed.
1	289	240	−17.0	1.62	8
2	321	258	−19.6	+	11
3	304	238	−21.7	2.61	13
4	295	227	−23.1	4.00	10
5	256	183	−24.6	1.26	13
6	282	212	−25.0	2.05	15
7	256	192	−25.0	1.81	13
Ave.: Pigeons which have lost 20% body weight.				2.23	
8	300	219	−27.0	1.15	15
9	304	218	−28.3	2.13	15
10	273	194	−28.9	3.41	13
11	316	221	−30.1	0	15
12	298	208	−30.2	0.35	10
13	335	202	−39.7	0	10
Ave.: Pigeons which have lost 30% body weight.				1.76	

As the above table indicates, the glycogen decreases at any stages of inanition and so it can be said that the mere inanition is quite different from polyneuritis as to the effect on the glycogen content in the liver.

(4) Liver Glycogen of Albino Rats fed on B_1-Free Synthetic Diet.

The typical symptom of polyneuritis was more frequently manifested by albino rats than by pigeons when fed on the following B_1-free synthetic diet: Fish meat protein 18, starch 68, butter fat 10, McCollum and Simmonds salt mixture 4. To this diet, 0.4 g autoclaved yeat were added. Young male rats weighing about 60 g were kept each in a separate wire cage and fed with the above diet and when the animals developed the disease, a part of them was cured by the administration of 4 mg active oryzanin daily. For control animals, 0.4 g dried yeast were supplemented to the B_1-free diet above mentioned.

Table 6. Liver Glycogen of Rats fed on B_1-Free Synthetic Diet.

Rat No.	Body weight, At the beginning of feeding. (g)	Body weight, At the appearance of the disease. (g)	Increase (+) or decrease (−) in weight. (%)	Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
1	56.0	42.5	−24.1	4.66	32	Spas.
2	61.5	58.5	− 4.9	8.02	33	〃
3	65.0	51 0	−21.5	8.04	34	〃
4	60.0	69.0	+15.0	6.13	36	〃
5	59.0	58.0	− 1.7	7.94	47	〃
6	56.5	54.0	− 3.5	6.96	37	〃
Ave.				6.96		
7	55.0	55.5	+ 0.9	3.65	31 (15;+17.0)	〃
8	56.0	56.0	± 0.0	3.82	33 (14;+ 8.5)	〃
9	57.0	55.5	− 2.6	1.82	32 (13;+17.5)	〃
Ave.: Rats cured by B_1-preparation.				3.10		

Table 7. Liver Glycogen of Rats fed on the Complete Synthetic Diet (Control).

Rat No.	Body weight, At the beginning of feeding. (g)	Body weight, At the end of feeding. (g)	Increase in weight. (%)	Liver glycogen. (%)	No. of days fed.
1	61.0	74.5	+22.5	4.96	14
2	56.0	80.5	+65.2	5.50	14
3	62.0	70.5	+13.7	5.08	14
4	62.0	97.5	+57.3	3.50	14
5	63.0	66.5	+ 5.6	2.88	15
6	67.0	106.0	+58.2	4.03	13
Ave.				4.33	

(5) Liver Glycogen of Underfed Albino Rats.

Rats were fed with the restricted amount of the basal diet above mentioned, supplemented with dried yeast and the glycogen content was estimated with the following results:

Table 8. Liver Glycogen of Underfed Rats.

Rat No.	Body weight. At the beginning of feeding. (g)	At the end of feeding. (g)	Decrease in weight. (%)	Liver glycogen. (%)	No. of days fed
1	65.0	57.5	−11.5	0.84	9
2	69.0	60.0	−13.7	1.77	10
3	70.0	60.0	−14.3	0	9
4	72.0	61.0	−15.3	0	15
5	77.5	65.0	−16.1	3.31	13
6	65.0	54.5	−16.2	0	15
7	75.5	62.0	−17.9	1.88	13
8	69.0	55.0	−20.0	+	19
9	80.0	63.5	−20.6	2.88	13
Ave.: Rats which have lost 10~20% body weight.				2.14	
10	65.5	47.5	−27.5	0.67	21
11	63.5	45.5	−28.5	0.77	21
12	71.0	50.0	−29.6	+	15
13	71.0	49.0	−34.0	0	19
Ave.: Rats which have lost 30% body weight.				0.72	

The above result is in full agreement with the 3rd. experiment on pigeons, i.e. those which have lost 30% of the original body weight contained only 0.72% in average.

(6) Liver Glycogen of Albino Rats fed on High-Fat Diets lacking both B_1 and Carbohydrates.

For the control, a complete high-fat diet was prepared as follows: Fish meat protein 29, lard 45, butter fat 20, McCollum and Simmonds salt mixture 6. To this, 0.4 g dried yeast were added daily.

The glycogen content was as follows:

Table 9. Liver Glycogen of Rats fed on the Complete High-Fat Diet (Control).

Rat No.	Body weight. At the beginning of feeding. (g)	At the end of feeding. (g)	Increase in weight. (%)	Liver glycogen. (%)	No. of days fed
1	57.5	104.5	+ 81.7	1.72	87
2	60.0	144.0	+140.0	2.16	87
3	58.5	129.5	+121.4	2.03	65
4	60.0	124.0	+106.7	1.82	65
Ave.				1.92	

The composition of B_1-free, high-fat diet used in this experiment was the same with the control diet except that 0.4 g autoclaved yeast were used instead of dried yeast. The animals fed on this diet developed typical avitaminosis more slowly and the liver glycogen was far less than those fed on the carbohydrate rich, B_1-free diet as shown in the following table:

Table 10. Liver Glycogen of Rats fed on B_1-Free, High-Fat Diet.

Rat No.	Body weight: At the beginning of feeding. (g)	Body weight: At the appearance of the disease (g)	Body weight: Increase (+) or decrease (−) in weight. (%)	Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
1	59.5	79.5	+33.6	1.91	32	Spas.
2	59.5	83.5	+40.5	2.39	70	〃
3	66.0	90.0	+36.4	1.66	65	〃
4	60.0	66.0	+10.8	1.40	66	〃
5	58.0	51.0	−12.1	1.70	35	〃
6	55.5	49.5	−10.8	1.53	47	〃
Ave.:				1.77		
7	60.5	53.0	−12.4	1.47	74 (6 ; + 2.0)	〃
8	67.0	69.5	+ 3.7	1.31	38 (16 ; +22.0)	〃
9	61.5	53.5	−13.0	1.43	41 (13 ; + 9.5)	〃
10	42.5	33.5	−21.2	2.09	31 (7 ; +11.5)	〃
Ave.: Rats cured by B_1-preparation.				1.75		

In the next experiment, the above high-fat, B_1-free diet was supplemented with absolute ethyl alcohol and saccharose respectively to the extent of 10%. In this case also the animals developed the disease but both alcohol and saccharose had apparenly no noticeable effect upon the glycogen content in the liver as shown in the following tables:

Table 11. Liver Glycogen of Rats fed on B_1-Free, High-Fat Diet added with 10% Alcohol.

Rat No.	Body weight: At the beginning of feeding. (g)	Body weight: At the appearance of the disease. (g)	Body weight: Increase (+) or decrease (−) in weight. (%)	Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
1	62.5	54.0	−13.6	1.20	63	Spas.
2	67.5	91.5	+35.5	1.15	71	〃
3	56.5	87.0	+54.0	1.61	75	〃
4	67.0	74.5	+11.2	1.46	77	〃
Ave.				1.34		

Table 11. Continued.

5	63.5	53.0	−16.5	1.60	42 (7 ;+17.5)	〃
6	61.0	66.5	+ 8.2	2.66	53 (7 ; + 8.5)	〃
7	63.5	73.0	+15.0	2.05	47 (12 ;+12.0)	〃
8	70.5	72.0	+ 2.1	2.19	67 (6 ;+16.5)	〃
9	58.0	80.0	+38.0	1.94	55 (14 ;+13.5)	〃
10	61.0	51.5	−15.6	1.19	74 (8 ;+ 9.5)	〃
Ave. : Rats cured by B_1-preparation.				1.94		

Table 12. Liver Glycogen of Rats fed on B_1-Free, High-Fat Diet added with 10% Saccharose.

Rat No.	Body weight. At the beginning of feeding. (g)	Body weight. At the appearance of the disease. (g)	Increase (+) or decrease (−) in weight. (%)	Liver glycogen. (%)	Days elapsed before the appearance of the disease.	Symptoms.
1	63.0	49.0	−22.2	1.73	32	Spas.
2	59.0	52.5	−11.0	+	33	〃
3	59.0	60.0	+ 1.7	1.99	54	〃
4	59.0	56.0	− 4.2	1.74	42	〃
5	62.5	45.5	−27.2	+	30	〃
Ave.				1.82		
6	66.5	69.0	+ 3.5	1.77	32 (9 ;+21.0)	〃
7	59.0	58.0	− 1.7	2.08	32 (9 ;+28.0)	〃
8	59.5	57.5	− 3.4	1.70	36 (9 ;+20.0)	〃
Ave. : Rats cured by B_1-preparation.				1.85		

Summary.

(1) The glycogen contents in the liver of pigeons and albino rats suffering from vitamin B_1-deficiency were estimated.

(2) In the case of pigeons, those which developed the disease without loosing much in body weight stored up more glycogen than the normal ones, i. e. 1.5 to 2.0 times higher than the latter, while those suffered from severe inanition and consequently heavily loss in body weight had always less glycogen than the control. In pigeons cured by the administration of active oryzanin (B_1-preparation), the liver glycogen was nearly normal.

(3) The underfed birds had always less glycogen than the normal ones.

(4) In albino rats fed on carbohydrate rich synthetic diet and developed the disease, the liver glycogen was without exception 1.5 times higher than the normal ones. In the animals cured by active oryzanin, the glycogen content was again reduced to the normal level.

(5) Though the rats fed on B_1-free, high-fat diet lacking carbohydrates have shown the typical symptom of avitaminosis, yet there was no increase of glycogen in the liver.

(6) It may be assumed that the abnormal increase of glycogen in the liver of avitaminous animals fed on B_1-free diet is a secondary phenomenon of the disease, but not the cause of it.

(7) As the diets used in these experiments contained B_2 in the form of autoclaved yeast, it can be concluded that B_1 has played an important rôle for the metabolism of liver glycogen.

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